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SOME ASPECTS OF CYTOLOGY IN RELATION TO THE STUDY OF GENETICS[1]

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THE consideration of genetic problems from the standpoint of cytological research sometimes encounters a certain opposition or prejudice which seems to me to be due to a misunderstanding of the position that is actually held by many cytologists. It probably grows primarily out of a conviction that the heredity of particular traits is not to be explained by referring them to the operation of particular cell-elements or "determiners," but results from an activity of the whole cell-system, or of the whole organism, regarded as a unit. With this view, as will appear, I am essentially in agreement. In the second place, the opposition is a kind of reaction or protest against the theory of pangens or biophores and the too elaborate logical constructions that have been built upon it, especially by Weismann. I also consider this theory untenable, or at least unnecessary. I will therefore attempt to outline a point of view from which I think genetic problems may reasonably be regarded from the standpoint of the cytologist.

The most essential result of modern genetic inquiry I take to be the proof of the independence of the so-called

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"unit-characters"—that is to say, that they may be independently combined, disassociated and re-combined in many different ways. The independence of these characters often seems to be complete; more rarely it is limited by definite phenomena of "coupling" or "repulsion." The interesting facts recently brought to light by Bateson and Punnett in case of certain unit-characters in plants, and by Morgan in case of sex-limited characters in flies, demonstrate that coupling or repulsion, as exhibited in the F_2 generation, are a consequence of an original association or separation in the grandparental gametes. In the cases referred to, characters that enter the F_1 zygote in the same gamete tend to "couple" (remain in association) in the gametes produced by this generation; while if these same characters enter the F_1 zygote in different gametes they tend to "repel" each other (remain separate) in the ensuing gamete-formation. This is almost a proof that the factors for coupled characters are borne by a common vehicle or substratum in the germ-cell, while in repulsion they are borne by separate ones. Not alone such facts, but the whole history of unit-characters points unmistakably to the conclusion that they are in some way connected with material substances or bodies; and that it is the combinations, disassociations and recombinations of the latter that explain the corresponding behavior of the former. For example, in sex-limited heredity the peculiar linkage of certain unit-characters with sex becomes readily intelligible, as several writers have recently pointed out, if factors necessary for the production of these characters are associated in the same material body with a factor that plays a certain necessary rôle in the production of sex. In this particular case, as it happens, we are actually able to see a material body (the "X-chromosome") which undergoes precisely such a mode of distribution with respect to sex and sex-limited characters as is demanded by the hypothesis. The question must here be squarely faced, in a very real and concrete form, whether

unit-characters are in fact dependent upon separate material bodies or substances, and whether the chromosomes can be regarded as such bodies or the carriers of such substances.

Without entering upon the evidence in detail I shall take it for granted that both these questions may be answered in the affirmative. Accepting this (if only for the sake of argument) how can such a conclusion be reconciled with the "action of the whole," and how formulated so as to escape the pangen-theory? The latter theory has fallen into discredit for two reasons. One is because of the quasi-metaphysical assumption that the "physical bases" or "determiners" of unit-characters are organized, self-propagating germs. Let us lay this assumption altogether aside as incapable of verification, and think of the "determiners" in a more vague way only as specific chemical entities of some kind. The second and more serious objection lies against the notion that the "determiners" are to be regarded as "bearers" of the corresponding characters. This is a fundamental error, as may be made clear, I think, by a specific illustration. It has been proved that many "unit-characters" are not units, but require for their production the cooperation of several factors, as is shown with especial clearness in the heredity of color. In such cases, as is now generally recognized, we should not speak of "unit-characters" but of unit-*factors*. Different "unit-characters" come into view as particular unit-factors are added to or subtracted from a given combination in the zygote. The factor for gray color in mice, to take a familiar example, operates by inducing a reaction of the germ that can only take place in the presence of several antecedent color-factors lower in the scale; and the latter, in turn, are only operative in the presence of still another factor that is necessary to the production of any color. The first and most obvious suggestion given by such facts is that what is added to or subtracted from a given combination or state of equilibrium in the zygote is some

kind of chemical entity which induces a specific reaction of the germ sooner or later in its development. But beyond this it is perfectly evident that however far backwards we may follow such a series of unit-factors, at every stage they play their specific rôle only in so far as they form part of a still more general apparatus of ontogenetic reaction that is constituted by the organism as a whole. *The whole of this apparatus, the entire germinal complex, is directly or indirectly involved in the production of every character.* We find it convenient, indeed necessary, to treat particular factors of reaction (*i. e.*, the "determiners") as if they were concrete and separate things. Such, in fact, they may be, as already indicated; but when we speak of them as "bearers" of the corresponding characters, we are using a figure of speech that may be highly misleading. The reactions (characters) which they call forth are not "borne by" them. They appear as responses of the germinal organization operating as a unit-system; and it is to this system as a whole that every character belongs, or by which it is "borne"—if indeed we may permit ourselves to employ the latter expression at all.

The point of view thus indicated may, I think, be made entirely clear by a chemical illustration. A number of writers, among them Adami, Guyer and Kossel, have of late called attention to the parallel that may be drawn between the physical basis of heredity and the complex molecular groups of the proteins and other organic compounds. It is a most suggestive one, though it is not to be taken too literally—indeed I shall employ it only as a kind of allegory or illustrative fiction. No one can fail to be struck with the really remarkable analogy, in method and in results, between the procedure of modern genetic experiment and that of modern organic chemistry. Just as the qualities of a particular protein may be definitely altered by the addition, subtraction or the substitution one for another of particular side-chains or molecular "Bausteine," so the addition, subtraction or

substitution of particular "determiners" or "factors" in the zygote calls forth specific responses that lead to the production of corresponding characters. The reasoning that applies to the first of these cases seems equally applicable to the second. No one, I suppose, would hold in the first case that the particular molecular groups or "Bausteine" concerned in the change are "bearers of" (*i. e.*, are alone responsible for) the resulting new qualities. The qualities of any protein, as Kossel has recently urged, belong to the molecule as a whole, and are not to be regarded as the sum of the qualities of its constituent "Bausteine." Why should we regard in a different light the "determiners" (chemical substances?) concerned in the second case? They are, clearly, not to be regarded as "bearers" or "physical bases" of the characters which depend upon their presence or absence. They are, I repeat, only differential factors of ontogenetic reactions that belong to the germ considered as a whole or unit-system.

In all this I am but expressing what I believe to be the point of view of many recent writers on genetic problems; but what I desire to emphasize is that the problems of cytology should be regarded from the same point of view. It is our task to see whether an apparatus of ontogenetic response can be discovered in the cell that fits with such a conception of the general process of determination. Is there cytological evidence of the existence in the germ-cell of such specific factors of reaction as I have referred to—in the nucleus, in the protoplasm, or in both? I think that observation and experiment alike have produced such evidence. Such experiments as those of Boveri on multipolar mitosis, and of Herbst and of Baltzer on the relations of the chromosomes in reciprocal crosses in sea-urchins have almost conclusively shown that the chromatin does in fact play a causative rôle in determination. Observation has gradually established the existence of a complex process of segregation and distribution of the nuclear materials in karyokinesis.

maturation and fertilization, that shows a most striking parallel to that of the factors of determination. That a somewhat similar apparatus of distribution may exist also in the protoplasm of the cell is indicated both by recent observations on the chondriosomes, or plastosomes, and by earlier results on experimental embryology. If in the brief discussion that follows I confine myself to certain phenomena of the nucleus it is because the history of the chromatin is more fully and accurately known.

The progress of cytological inquiry tends steadily, I think, to sustain the view first clearly formulated by Wilhelm Roux that the nucleus contains many different substances which undergo orderly groupings and distributions in the karyokinetic phenomena. These processes are in some measure made visible to us in the formation of spireme-threads, in their history in cell-division, and in the still imperfectly understood but perfectly definite events of synapsis and reduction. In his well-known paper on the significance of the karyokinetic figure, published in 1883, Roux maintained that the nucleus is the seat of many different "qualities." He committed himself to no definite view as to what these "qualities" really are; but the implication is not far to seek that they have a chemico-physical basis and may be different chemical substances. On this general assumption he based his well-known interpretation of karyokinesis, of which the essential postulate was that the "qualities" (substances?) become arranged in linear series in the spireme-thread, and by longitudinal splitting of the thread may thus be equally divided (or otherwise definitely distributed) to the daughter-nuclei. I believe that many of the later advances of cytology lend additional support to this conception.

1. In the first place, the evidence gives strong ground for the conclusion that the chromosomes, to which the spireme-thread gives rise, are not homogeneous, but compound bodies. I do not here refer to the well-known fact

that the spireme-threads often consist of linear series of granules. I have in mind the fact that the number and size-relations of the chromosomes often differ materially as between different species, even nearly related ones, and that in at least one case (that of the X-chromosome) it is an established fact that a particular chromosome which in some species is a single body may be represented in other species by two or more components that sometimes show constant and characteristic differences of size. The natural interpretation of this fact is that the chromosomes are compound bodies, consisting of different constituents which undergo different modes of segregation in different species. We may here find a rational explanation, both of sex-limited heredity, as I have elsewhere indicated, and of other kinds of coupling.

2. Recent studies on karyokinesis and maturation emphasize anew the importance of the mitotic transformation of the chromatin-substance, and add weight to Roux's original interpretation of this phenomenon. Nothing in recent cytological research is more interesting than the discovery by Bonnevie, Pinney, Davis, and others that new chromosomes may arise within the old ones in the form of tightly coiled or convoluted threads, which uncoil or unravel to form separate spireme-threads. In karyokinetic division these threads may be formed already inside the telophase-chromosomes of the preceding division, as was discovered by Bonnevie. In other cases, an example of which is given by certain Orthoptera first reported upon by Miss Pinney, they are first visible in the early prophases, when they are seen uncoiling from massive bodies formed from the old chromosomes and equal in number to them. The same remarkable process occurs in the early auxocytes, as the chromosomes are preparing for conjugation in synapsis, as has been shown particularly by Davis, whose observations, like those of Pinney, I have recently been able to confirm and extend. In many insects the presynaptic spireme-threads do not arise, as has often been described in other

forms, directly from a chromatin-network. They arise from massive bodies, each of which resolves itself into a closely convoluted thread which then uncoils before conjugation takes place. Why should chromosomes that are already formed as massive bodies delay their division or conjugation until so remarkable a redistribution of their substance has taken place? It is not a necessary condition of conjugation, as is proved by the case of both the sex-chromosomes and the *m*-chromosomes, which do in fact conjugate in the massive condition. All the facts become intelligible in the light of Roux's hypothesis that the formation of the spireme-threads effects a linear alignment of different constituents in preparation either for division or for a definite type of association in pairs in synapsis.

One of the most interesting applications of this view to genetic phenomena is that suggested by Janssens in his theory of the "chiasmatype," which has recently been applied by Morgan to the explanation of coupling and repulsion. In the twisting together of the spireme threads, either in synapsis or at a succeeding stage, and the subsequent secondary splitting of the thread in one plane is provided a very simple mechanical basis, both for the free interchange of factors between the homologous chromosomes and for the phenomena of coupling or repulsion, which are otherwise so difficult to comprehend. I do not maintain that this particular interpretation, or the more general one of Roux, is demonstrably true, or that no other explanation can be found. I only hold that they are legitimate conceptions which may be tested by observation and experiment, and which must be fully reckoned with as intelligible interpretations of the facts before we can set these facts aside as utterly mysterious or as a meaningless by-play.

3. I would lastly recall the experimental proof by Boveri that the chromosomes differ among themselves in their physiological relation to development, and the corresponding cytological fact that they differ among them-

selves also in respect to size, behavior, or both. In one case only has it thus far been possible to demonstrate a constant relation between particular chromosomes and particular characters, namely in the case of sex and sex-limited characters. It is true that in this case we are not able to assert that the sex-chromosomes are the primary determining cause of sex—indeed, there seems to be good evidence to the contrary. Unless, however, we are prepared to defend the proposition that the sex-chromosomes are absolutely functionless we shall not, I think, escape the conclusion that they form one of the factors in sex-heredity.

I will not enter upon the analytical subtleties of the problem whether the chromosomes, or the substances that they contain, are permanent and self-propagating elements or are merely temporary products of an unseen underlying activity—whether they are causes, effects or mere accompaniments of the specific reactions with which they are somehow connected. These are fundamental questions; and some of them can not yet be answered. But we should not hesitate to adopt what seems likely to be for the time the most reasonable and fruitful working view. "Hypotheses," said Pasteur, "come into our laboratories by armfuls; they fill our registers with projected experiments, they stimulate us to research—and that is all." In my view studies in this field are at the present time most likely to be advanced by adopting the comparatively simple hypothesis that the nuclear substances are actual factors of reaction by virtue of their specific chemical properties; and I think that it has already helped us to gain a clearer view of some of the most puzzling problems of genetics. But even if we adopt the opposite view that the formation, segregation and distribution of these substances are only signs or indices of what lies behind them, we still have in this direction one of the most promising paths of approach to a study of the activities of the germ-cells in heredity.

It will perhaps be said that such conceptions of the

nuclear organization as have here been indicated are both vague and artificial. Vague and crude they undoubtedly are; and so they will remain until we have far more thoroughly explored a field of inquiry in which we must for the present make a shift with crude ideas unless we are content to work with no ideas at all. They may be artificial too; but it appears to me that in this respect they differ only in degree from the graphic formulas of structural chemistry. The chemist does not hesitate to picture definite topographical or spatial relations in the complex organic molecule—symmetrical and asymmetrical forms, cyclic or ring-formations, linear series, side-chains and other such graphic constructions. It is by their use that the whole science of organic chemistry has been built up, and that such men as Emil Fischer and Kossel have made nearly all of their advances in our knowledge of those most complex of known organic compounds the proteins. And these constructions are regarded by eminent investigators as something more than mere figurative expressions or symbols. They are taken more literally as representations or models—rude, no doubt, but as far as they go real—of the actual arrangements in space of the various molecular groups or protein "Bausteine." If therefore observation and experiment lead the cytologist to postulate definite topographical relations among the nuclear substances, and if such a conception help him to explain the results of genetic studies, he finds himself in good company, even though his present clumsy notions regarding the nuclear organization can as yet make no approach to the exact and elegant constructions of the chemist.

The essential conclusion that is indicated by cytological study of the nuclear substance is that it is an aggregate of many different chemical components, which do not constitute a mere mechanical mixture, but a complex organic system, and which undergo perfectly ordered processes of segregation and distribution in the cycle of cell-life. That these substances play some definite rôle

in determination is not a mere assumption, but a conclusion based upon direct cytological experiment, and one that finds support in the results of modern chemical research. Professor Kossel has recently said that every peculiarity of the species and every occurrence affecting the individual may be indicated by special combinations of protein "Bausteine." The point of view that has here been indicated is entirely in accordance with such a conception. The results of cytological inquiry fit with the view that there are many such combinations in both nucleus and protoplasm; and the interest of cytological study lies in the fact that we can in some degree follow out their modes of segregation and distribution with the microscope. We are still utterly ignorant as to how these processes are determined; and the more one studies them the more one's wonder grows. I would certainly be one of the last to disparage the brilliant results that have been attained through the prolonged and patient labors of cytological observation and experiment. They stand, I believe, among the most interesting and valuable achievements of modern biology. But these studies have as yet made no approach to their limit, and a vast unexplored field still lies before us. We may as well recognize the fact that our present rude notions of cell-organization have not yet progressed very far beyond the paleolithic stage of culture; but they are of use in so far as they help to open new points of view or to discover new facts, whether in cytologic or in genetic inquiry. It seems to me that in both regards they have already proved worth while.

THE CORRELATION BETWEEN CHROMOSOMES AND PARTICULAR CHARACTERS IN HYBRID ECHINOID LARVÆ[1]

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THE student of genetics who bases his researches on the development of echinoderms has become quite accustomed to the criticisms of his friends to the effect that his work can have little point until he can show them individuals of a second or a third generation, and he receives these criticisms at their full value.

Our ignorance on this subject has been clearly defined. First, little is known of the inheritance in the adult hybrids of the characters of the parent species, and second, it is not known that these hybrids become sexually mature.

The ready acceptance of these ideas has blinded us, in a measure, to the fact that for the solution of some of the problems of heredity, the echinoderms offer material of unusual advantage. The plants, insects and vertebrates are peculiarly adapted for the elucidation of the later aspects of heredity; the echinoderms are available for information concerning the processes which occur during and immediately after fertilization.

In the course of this paper I shall show that we are now in a position to predict certain characters in the adult hybrids from a knowledge of the germ cells of the ancestors and, granted the sexual maturity of the hybrid adults, to predict the character of their germ cells.

The evidence that I shall bring forward is based on a study of chromosomes. The advantages afforded by echinoderms for such a study, lie in the fact that not only may we study the chromosomes during the divisions of

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the normally fertilized egg, but we may study the chromosomes of the egg itself, *i. e.,* the maternal chromosomes, in artificially parthenogenetic eggs; we may study the chromosomes of the spermatozoan, *i. e.,* the paternal chromosomes, in fertilized enucleated egg fragments; and we may study the chromosomes in cross-fertilized eggs, identifying here those which have come from one parent or the other.

The first subject of which I shall speak is the correlation of certain chromosomes with sex.

The conclusions reached by Baltzer, from a study of *Echinus* and *Strongylocentrotus,* are known to most of those present. They find their readiest expression in the statement that "in Echinoids the female is digametic while the male is homogametic." I have expressed elsewhere the idea that this generalization is too broad and that the statement should be limited to the cases for which the condition was described; but I must do more than this. I can admit that apparent condition for but one of Baltzer's cases, that of *Echinus,* for his own illustrations for *Strongylocentrotus* indicate that this form is in agreement with another interpretation, and not with the one given.

In the forms which I have studied, the male is digametic, the female homogametic, as in the insects.

The first case that I shall submit is that of *Tripneustes esculentus* (*Hipponoë*), in which the evidence is especially clear, although not submitted to the ultimate analysis that has been given to the second case.

In *Hipponoë,* I have been able to show, by the study of cross-fertilized eggs, made in connection with straight fertilized *Toxopneustes* and *Hipponoë* eggs by two of my students, Miss Heffner and Miss Pinney, that a chromosome of peculiar form, namely hook-shaped, is transmitted by half of the *Hipponoë* spermatozoa, and that the eggs which receive it must become males.

The evidence here is clear. Such an element never occurs in straight-fertilized *Toxopneustes* eggs. It is

found in one-half of the straight fertilized *Hipponoë* eggs; it is found in half of the *Toxopneustes* eggs which have been fertilized by *Hipponoë* sperm, and it never occurs in *Hipponoë* eggs fertilized by *Toxopneustes* sperm.

There is then absolutely no appeal from the fact that in *Hipponoë* the spermatozoa are of two classes, one with, the other without, this idiochromosome.

The second case that I shall submit is that of *Lytechinus,* better known to us as *Toxopneustes.* Here I am able to give evidence gained by a study of straight fertilized eggs, artificially parthenogenetic eggs, fertilized enucleated egg fragments, and four crosses, the reciprocal *Hipponoë* × *Toxopneustes* and *Arbacia* × *Toxopneustes* crosses.

The study of straight fertilized *Toxopneustes* eggs showed two classes of zygotes, one with two V-shaped chromosomes, the other with three. This seemed in agreement with conditions in *Echinus,* although obviously there was no chance of tracing the source of the heterochromosome in such material.

Those who have devoted any attention to the chromosomes in Echinoids know the difficulties involved in such a study. The chromosomes are all small; most of them have the form of short, straight or slightly bent rods, while but three or four, at most, may be distinguished by peculiarity of form. Difficulties attend the study of even these chromosomes. The V's in *Toxopneustes,* for example, best seen in the anaphases of division, may have both arms brought into contact and so resemble a single, somewhat thickened rod.

Since this is true, it should be easier to determine size differences when only the haploid number is present than when the full number of chromosomes is in the division figure.

So far as I am aware, none of the cytological work on artificial parthenogenesis in Echinoids has been done with the idea of an individuality of form of chromosomes

in mind. The chief aim has been the determination of the presence of the haploid number of chromosomes and of the fact that auto-regulation does not occur. This is true for results published as late as 1910.

In my study of this material I have found that all of the eggs are alike, possessing among the others two V-shaped chromosomes.

In the fertilized enucleated egg fragments, two classes are evident. Half of the spindles show two V-shaped chromosomes in each anaphase plate while half show but one. I have no conclusive evidence for showing whether we are dealing with an *X* chromosome or with *X* and *Y* chromosomes. Counts are decisive for either view, and therefore valueless. I believe that this is a matter which can be decided only by a study of the spermatogenesis of these forms.

For some reason there is a belief in the tradition that in Echinoids the somatic number of chromosomes is thirty-six and in one case eighteen. Counts of perfect polar views of anaphase plates in *Toxopneustes* give the numbers thirty-seven and thirty-eight, but even from these I do not feel warranted in applying a sex formula.

It will be noted that the number of V's does not agree with our expectation from the straight-fertilized eggs. I can only say that my description is of the facts as I find them in my preparations. From this it would seem that the number of V-shaped chromosomes in straight-fertilized *Toxopneustes* eggs should be three and four. These facts are further substantiated by conditions in the *Hipponoë* × *Toxopneustes* crosses.

The second point of which I wish to speak is that of correlation of chromosomes and larval characters. The only larval structure which has a form sufficiently definite to be used for comparison is the skeleton. The most advantageous forms for this purpose are those in which the pluteus of one parent has skeletal arm rods of a simple straight form and the other rods of the latticed type.

It is well known that an apparent confusion exists among observations on hybrid Echinoid larvæ, as to whether plutei of a maternal type, a paternal type or of mixed form are derived from certain crosses. Different results have been obtained by different investigators and by the same investigators working in different regions, or in the same region in different seasons.

From my own results I am convinced that there is no reason for believing that any of these observations are incorrect and that this dissimilarity of results may be traced to the influence of the constitution of the sea water at different times and places, to temperature, etc.

If we look into the matter carefully we find that there is a correlation between the fate of the maternal and paternal chromatin and the character of the plutei.

It is a remarkable fact that in the general literature of genetics, authors who have noted the fact of elimination of chromosomes, as shown by the researches of Baltzer and Herbst, have not also noted their retention in some cases, and the influences of this retained chromatin on the character of the larvæ.

It may be seen, by the examination of Baltzer's tabulation, given in his 1910 paper, that in the cases cited an elimination of presumably the introduced paternal chromosomes is followed by a pluteus of the maternal type, and that the retention of all the chromosomes may be followed in some cases by a pluteus of a mixed or intermediate type, in other cases by a pluteus of the maternal type. To these cases I must add the case of the retention of chromosomes followed by a pluteus of the paternal type, this being afforded by the *Hipponoë* ♂ × *Toxopneustes* ♀ cross.

In this instance we have a clear example of *Hipponoë* dominance with respect to larval skeletal character, and this dominance is correlated with the retention of *Hipponoë* chromosomes.

I have not asserted that every *Toxopneustes* egg which is fertilized by *Hipponoë* sperm gives rise to a pluteus

with a *Hipponoë* skeleton. The largest percentage of the eggs does respond in this manner; a smaller percentage dies in the blastula and gastrula stage, and a still smaller percentage shows little or no trace of paternal influence.

If we take the eggs of such a fertilization and examine them during the segmentation stages, we shall see that in these eggs the greatest number show normal division figures, and by normal I mean those of the almost diagrammatic sort which may be seen in straight-fertilized *Toxopneustes* eggs, figures which give no indication of any chromosome elimination, while the smaller number show aberrant figures with varying degrees of elimination.

The normal division figures correspond in number to the plutei with the paternal type of skeleton and the aberrant figures to the plutei of intermediate and maternal character.

Clearly, then, in the hybrids of Echinoid crosses we may have dominance of one species or the other with respect to the skeleton, and this dominance may be transmitted by the egg or by the spermatozoan. Of the considerable number of crosses that I have made, only one, the *Hipponoë* ♂ × *Toxopneustes* ♀ cross gives this evidence.

Some facts of interest are demonstrated by the *Arbacia* × *Toxopneustes* crosses. These crosses are made with difficulty and I had never succeeded in getting them until the past summer. The chromosomes in *Arbacia* are much smaller than those in *Toxopneustes* and I had hoped that material from this cross would be of use when compared with my experimental *Toxopneustes* material. Here, however, I found that there is an elimination of chromosomes from the first, an elimination which may involve the rejection of not only the foreign chromosomes, but some of those of the egg as well, the result being that, in some instances, the full haploid number of neither is retained, and few zygotes pass through the blastula stage.

A further insight into the effect of the retention of foreign chromatin is given by partially arrhenokaryotic and partially thelykaryotic plutei. In such larvæ, with their asymmetrical bodies and skeletons, we may distinguish regional differences by nuclei of different sizes. and by a study of segmenting eggs we may find how these nuclei have arisen. In some instances we know that the fertilization processes have been so modified that subsequent divisions of the egg contain only paternal nuclear material and are paternal in character. In other regions part or all of the foreign chromatin is present and has modified development.

The conditions noted in the plutei find correlation in the following conditions in the egg.

1. Retention of all chromosomes and dominance of one species over another with respect to skeletal characters.
2. Elimination of part of the chromatin and dominance of one species over another with respect to skeletal characters.
3. Elimination of part of the chromatin and intermediate skeletal characters.
4. Elimination of part of both paternal and maternal chromatin and inhibition of development.

The first three, at least, may all occur in a given lot of eggs, and, since this is true, depend in part on chance, just as we may have imperfect mitotic figures in straight-fertilized eggs.

The second and third cases may indicate an incompatibility between the chromosomes and the cytoplasm of the egg; the fourth case, not this alone, but an incompatibility between the chromosomes themselves.

Finally as to our ability to predict the character of the adult from the characters of the larvæ.

It is clear that in *Hipponoë* and *Toxopneustes* we may predict sex, since we have seen that maleness and a heterochromosome of paternal origin are correlated. It is thus evident that if chemically fertilized *Hipponoë* and *Toxopneustes* eggs develop to sexual maturity these in-

dividuals will be females, the reverse of our belief concerning the naturally parthenogenetic egg of the bee. In cases similar to those described by Baltzer for *Echinus,* half the adults will be males and half females.

A prediction as to other characters is difficult to make, for the fact that one species is dominant over another with reference to larval skeletal characters give no idea as to other characters.

Nevertheless, in the cases of partial arrhenokaryosis which I have mentioned we should expect a region of the larval body containing only paternal nuclear material to place its stamp on that part of the adult body arising from it. A natural hybrid showing pure regional resemblances to one species or another is theoretically possible and would find its explanation in partial arrhenokaryosis.

For the inheritance of most characters we must fall back on our knowledge of natural echinoderm hybrids, which is increasing steadily.

We should expect that where parts of both contributions of chromatin have been retained and an intermediate pluteus has resulted the adult will be of the mixed type. One of the supposed Echinoid hybrids described by Mortensen fulfils this expectation.

The proof of these predictions lies in the rearing of hybrid larvæ to the adult condition. This we know to be possible. It requires only laboratory advantages of an unusual type, care, time and an accurate knowledge of the natural history of echinoids.

DARWIN'S THEORY OF EVOLUTION BY THE SELECTION OF MINOR SALTATIONS

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THERE is an opinion which is becoming more widely prevalent daily that Charles Darwin's theory of selection rests upon "fluctuations" which may not be heritable. Nothing could be further from the facts.[1]

It is true that Darwin finally came to believe in the inheritance of somatic modifications (in the modern sense of bodily changes) caused by the direct action of environment as well as by habit (ontogeny), but in his original (1859) and final (1872) opinion evolution was *chiefly* due to the selection of heritable "individual differences."

Darwin's true meaning as to "*individual differences*" is not to be found in his language, but in the *cases* he cited; he has been widely misunderstood[2] as believing in continuous evolution whereas he chiefly believed in discontinuous evolution.

Darwin's final opinion[3] may be cited with a transposi-

[1] De Vries is partly responsible for this general misunderstanding of Darwin. In "Die Mutationstheorie," Leipzig, 1901, pp. 21–27, we find a very full discussion of the opinions of Darwin in which the interpretation is reached that Darwin was not clear in distinguishing "variation" and "mutation."

[2] Cox, Chas. F., "Charles Darwin and the Mutation Theory," *Ann. N. Y. Acad. Sci.*, Vol. XVIII, No. II, Pt. III, February 10, 1909, pp. 431–451. This is a most conscientious and exhaustive review of Darwin's opinions in which the following conclusion is reached: "As we have seen, he was compelled to concede that what we now call mutation had occasionally taken place and become the starting point of new races, but he was none the less unshaken in the conviction that this process was exceptional and extraordinary, and that, as a rule, a new species originated by the gradual building up of minute and even insignificant deviations from the average characters of an old species, *which deviations we now call fluctuations.*" [P. 450, italics our own.]

[3] "The Variation of Animals and Plants under Domestication," Vol. II, p. 397.

tion of sentences and italicizing of important words, as follows:

> . . . we have abundant evidence of the constant occurrence under nature of slight individual differences of the most diversified kinds; and we are thus led to conclude that species have generally originated by the natural selection of extremely slight differences. This process may be strictly compared with the slow and gradual improvement of the racehorse, grayhound, and gamecock. As every detail of structure in each species has to be closely adapted to its habits of life it will rarely happen that one part alone will be modified; but as was formerly shown, the co-adapted modifications need not be absolutely simultaneous. Many variations, however, are from the first connected by the law of correlation. Hence it follows that even closely allied species rarely or never differ from one another by one character alone . . . from the history of the racehorse, grayhound, gamecock, etc., and from their general appearance we may feel nearly confident that they were formed by a slow process of improvement; and we know that this has been the case with the carrier-pigeons as well as with some other pigeons. . . . It is certain that the ancon and mauchamp breeds of sheep, and almost certain that the niata cattle, turnspit, and pug dogs, the jumper and frizzled fowls, short-faced tumbler pigeons, hook-billed ducks, etc., suddenly appeared in nearly the same state as we now see them. So it has been with many cultivated plants. The frequency of these cases is likely to lead to *false* belief that natural species have often originated in the same abrupt manner. But we have no evidence of the appearance, or at least of the continued procreation, under nature, of abrupt modification of structure, and various general reasons will be assigned against such a belief.

Thus Darwin, on the admirable ground that *no evidence had been adduced in nature* of such transformation, rejected the hypothesis of major saltatory evolution as causing the *natural* appearance of entirely new types of animals or plants or of entirely new and profoundly modified organs. There was no evidence in 1872 and there is none to-day of the sudden appearance in nature of such a breed as the ancon sheep. On the other hand, Darwin's meaning as to "*slight individual differences of the most diversified kinds,*" as clearly conveyed in the hundreds of observations he cited in "The Origin of Species" (edition of 1872) and especially in his "Variation of Animals and Plants Under Domestication," is

that such individual differences were in the nature of minor saltations of character, structural or functional, and always hereditary. Since Darwin observes that *natural selection may be strictly compared with the slow and gradual improvement of the racehorse, grayhound,* etc., if we collect all the observations which he assembled as to the genesis of the racehorse and grayhound we may gain a concrete understanding of his opinions as to the genesis of species. It will appear that many of the *new characters* which Darwin cites, and he used the term "new characters" interchangeably with "individual differences," are clearly identical with minor saltations or with the "mutations of De Vries," as generally understood by zoologists to-day.

"We may therefore conclude," observes Darwin,[4] "that, whether or not the various existing breeds of the horse have proceeded from one or more aboriginal stocks, yet that a great amount of change has resulted from the direct action of the conditions of life, and probably a still greater amount from the long-continued selection by man of slight individual differences." Among the examples he cites in amplification of this conclusion are the following:

(1) Eight incisors or two super-normal; (2) canines in females; (3) a nineteenth or posterior rib; (4) a supplementary hock-bone; (5) a reversional trapezium and Mtc.V; (6) horn rudiments in the frontal bones one or two inches in length; (7) tailless foals which, he observes, might have produced a new breed; (8) curled hair correlated with short manes and tails and mule-like hoofs.

All the above "individual differences" are cited by Darwin as hereditary; all are "discontinuous" in Bateson's sense or "mutations" in De Vries's sense. In other parts of Darwin's works most of the numerous references made to "individual variations" in horses are of the same character, namely, saltatory and hereditary. Although (5) cited above is a "reversion," reversions and

[4] "Animals and Plants under Domestication," Vol. I, p. 55.

ontogenic modifications are, for the most part, clearly distinguished as belonging to another category.

Similarly Lutz[5] has kindly analyzed for the writer twenty-one cases cited by Darwin among insects as possible material for selection and as indicating modifications by environment. Eight of these cases are doubtful; seven cases represent "gradations," or "slow degrees," probable or possible continuity; six cases represent discontinuity. It seems to Dr. Lutz that Darwin made the most of the cases of discontinuous variation he knew about, but that for the most part he had continuous variation in mind.

It is therefore of interest, in view of the neglectful and almost contemptuous attitude of certain writers toward Darwin's observations, to make a fresh summary of the principles found scattered through the pages of his great work on "Variation," reexpressing some of these principles in modern terms.

Darwin on "New Characters" and "Individual Differences"[6] as Expressed in Modern Terms

(1) Newly appearing characters arise from unknown causes, either stable in heredity, variable in heredity, or not hereditary at all.[7] (2) "Individual variations" are minor suddenly appearing characters, heritable. (3) Characters of all kinds, whether new or old, tend to be inherited. Those which have already withstood environment will, as a rule, continue to withstand it and be truly transmitted. (4) A change of environment is principally but not invariably the source of new variations. (5) There are periods of variability or mutability in which many new characters appear. (6) New characters are observed to accumulate in successive generations in the

[5] Frank E. Lutz, letter to the writer, December 15, 1911, "References to insects in the 'Origin of Species,' especially those bearing on the question of continuity *versus* discontinuity."

[6] See American edition, 1900, "Animals and Plants under Domestication," dated by Darwin January, 1872.

[7] Vol. II, p. 37.

same direction. (7) There is a predisposition to similar progressive variations in descendants of one stock (termed "analogous or parallel variation") due to unknown causes acting on similarly constituted organisms.[8] (8) There is a marked prepotency in heredity in saltatory types, *e. g.*, ancon sheep, turnspit dogs. (9) There is "in a certain sense" a mosaic inheritance (after Naudin) observed in hybrids, or character segregation by self attraction and self affinity. Similarly there is an exclusive inheritance, as observed in characters derived exclusively from the father or mother and lying dormant or latent for many generations. (10) There is a particulate inheritance, for example, of the special quality of vigor and endurance observed in descendants of the racehorse "Eclipse"; *i. e.*, a dominance of single characteristics. (11) A large number of individual variations cited are antithetic characters rather than slight or infinitesimal gradations of similar characters, *e. g.*,

Erect ears	Drooping ears
Fertility	Sterility
Immunity	Non-immunity
Resistance to environment	Non-resistance to environment

(12) Environment directly affects the antithetic characters of sterility and fecundity. (13) New characters may appear and old ones disappear at any stage of development.[9] (14) There is a similar age heredity or inheritance at corresponding periods of life. (15) There is a sex-limited heredity. (16) There is a correlated variability or coupling of new characters in heredity, *e. g.*, black color of the skin and immunity from disease. (17) Particulate inheritance as shown in non-blending colors, *e. g.*, gray and white mice produce piebald young, or gray or white young, but never a blend between gray and white. (18) Old characters lying latent are ready to be evolved under certain old environmental conditions. Thus we observe the ontogenic revival of feral characters

[8] Vol. II, p. 329.
[9] Vol. II, p. 392.

in feral environment, and of domesticated characters in return to domestication, *e. g.*, barking in dogs. (19) Ontogenic reactions to environment are observed in all external and internal characters in animals and plants. (20) Under *selection* Darwin anticipated the theory of organic or coincident selection.[10]

Summary

Darwin did not so sharply distinguish in *language* as Weismann and Mendel have taught us to between the different kinds of variation and different factors of evolution, yet in his observations and the cases he cites his perception is absolutely clear between *heredity, ontogeny, environment* and *selection* as interoperating factors. In fact, he remarks[11] on the frequent difficulty of distinguishing between the inextricably mingled factors of ontogeny (*e. g.*, effects of use and disuse), and of correlated or coadapted variability and of spontaneous variations. A comparison of all the various kinds of variation cited by Darwin in his two great volumes shows that they fall into the following four classes:

I. "Individual variations," "spontaneous variations," new suddenly appearing heritable characters, practically equivalent to the minor mutations of De Vries, believed by Darwin to be the chief material of natural selection and evolution.

II. Sports, or major saltations, such as the "mauchamp," "ancon" and "niata" breeds, believed by Darwin not to occur in a state of nature.

III. Fluctuations of proportion, congenital and hence transmissible, equivalent to the quantitative variation of Bateson, best illustrated by Darwin in his theory of the evolution of the long neck of the giraffe:

> So under nature with the nascent giraffe, the individuals which were the highest browsers, and were able during dearths to reach even an inch or two above the others, will often have been preserved; for they

[10] Vol. II, pp. 317, 318.

[11] Vol. II, p. 327.

will have roamed over the whole country in search of food. That the individuals of the same species often differ slightly in the relative lengths of all their parts may be seen in many works of natural history, in which careful measurements are given. These slight proportional differences, due to the laws of growth and variation, are not of the slightest use or importance to most species. But it will have been otherwise with the nascent giraffe, considering its probable habits of life; for those individuals which had some one part or several parts of their bodies rather more elongated than usual, would generally have survived. These will have intercrossed and left offspring, either inheriting the same bodily peculiarities, or with a tendency to vary again in the same manner; whilst the individuals, less favored in the same respects, will have been the most liable to perish.

IV. Fluctuating variability, clearly distinguished by Darwin from II and not especially connected by him with the process of evolution.

December 30, 1911

THE COLOR SENSE OF THE HONEY-BEE: THE POLLINATION OF GREEN FLOWERS[1]

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CARL NAGELI, in a memoir often cited, after stating the common opinion that insects are especially attracted by the bright coloration of floral organs, says: "We understand now why there are no green flowers; they would be invisible in the midst of foliage."

We seem to dream when we read these lines written seriously by a naturalist. As the lists below demonstrate, not only are there a large number of green or greenish flowers, invisible or scarcely visible in the midst of foliage, but insects discover them without the least difficulty.

This false notion of the non-existence of green flowers, which can only be excusable among those unacquainted with botany, has arisen from many causes; the plants which bear flowers of this nature are only cultivated when they are of medicinal use or are valuable for food, when they are frequently regarded as vegetables rather than as flowers. In foreign countries natural history collectors travelling for horticulturists disdain everything which has not at least a decorative foliage. Furthermore, the authors who attach great importance to the coloration of the floral envelopes for the attraction of insects have often neglected to mention in their works that such and such a species, though fertilized by winged arthropods, possesses green or greenish flowers. H. Müller has committed this fault many times, and Charles Darwin in his celebrated work on the fertilization of orchids passes over the color usually in silence.

A reaction was necessary; it was important to remind biologists that there exists a long series of green flowers, greenish, or scarcely visible, which insects find as easily as the others, despite the absence of their so-called attractive colors.[2]

Plateau then proceeds to enumerate 91 entomophilous species, of which 41 had green or largely green flowers,

[1] "The Color Sense of the Honey-bee: Is Conspicuousness an Advantage to Flowers?" AMER. NAT., 43, 338–349, June, 1909; "The Color Sense of the Honey-bee: Can Bees Distinguish Colors?" AMER. NAT., 44, 673–692, November, 1910.

[2] Plateau, F., "Comment les fleurs attirent les insectes," 4me partie, *Bull. Acad. roy. Belgique,* 3me série, 34, 614–615, 1897. In the writer's opinion, whenever Müller or Darwin omits to mention the color of a flower, it is either through inadvertence or because it is assumed to be well known.

38 greenish and 12 brownish or brown flowers, to the larger part of which insect visits had been observed by himself or other floroecologists. Of 72 of these forms he examined the color himself and observed insect visits to 63 of them, or more than two thirds. Plateau believed that the visits of insects to green flowers very strongly supported his views, and in one of his last papers on the pollination of *Listera ovata*, an orchid with green flowers, he again returns to this subject. In a letter to the writer he states that he had made innumerable observations on the pollination of green flowers, which had extended over twelve years. His conclusion as finally expressed is as follows:

> It is not the colors more or less bright of the corollas but other causes which guide to flowers their winged pollinators. Green or greenish flowers notwithstanding their hue similar to that of foliage are as effectively fertilized by insects as white, blue, red, or yellow flowers, consequently, all these floral colorations might disappear from nature without the pollination and reproduction of plants being diminished.[3]

It is desirable to examine briefly the pollination of green flowers and to consider whether this conclusion is sustained or not.

A familiar example of a yellowish-green flower attractive to insects is offered by the garden asparagus (*Asparagus officinalis*). The flowers are mellifluous, pleasantly scented and frequented by honey-bees and less often by smaller species of bees. Müller says that "in spite of their inconspicuous color they are easily visible at a distance"; but Plateau describes them as "peu visible," an illustration perhaps that an observer is apt to be influenced by his point of view as well as by the color of the flower. As the result of personal trial I find that they can be distinctly seen at a distance of at least fifty feet. Another yellowish-green flower is *Tilia ulmifolia*, or basswood, which, according to both Müller and Plateau, is sought by honey-bees in immense numbers. This is also true of the American basswood (*Tilia ameri-*

[3] Plateau, F., "La pollination d'une orchidée a fleurs vertes '*Listera ovata* R. Br.' par les insectes," *Bull. Soc. roy. Belgique*, 46, 339, 1909.

cana), which, according to Root, an eminent authority on American apiculture, furnishes more honey than any other plant known, with perhaps the single exception of the white clover.[4] Other greenish, or dull-colored flowers, which the honey-bee was observed to visit were *Ampelopsis quinquefolia, Teucrium Scorodonia, Comarum palustre, Atropa Belladonna,* and several species of *Scrophularia.*

But of the 91 greenish or brownish hued species the honey-bee was seen to visit only 27, that is, there are no recorded visits of this bee to a little over seventy per cent. of the listed flowers. To four species there are no records of the visits of any insect. The remaining species were chiefly visited by flies, sometimes minute species, beetles, and the less specialized Hymenoptera; but *Hedera helix* was attractive to wasps and *Platanthera bifolia* to Lepidoptera. How meager the number of visits was should be noted: *Celastrus Orixa* was visited only by the domestic fly and one other species of Diptera; *Alchemilla hybrida* only by small Muscidæ; *Alchemilla alpina* in the Alps by one beetle and two Muscidæ; *Alchemilla fissa* in the same mountains by three Muscidæ; the sessile green flowers of *Herniaria glabra* were visited only by very small insects; the yellowish-green flowers of *Amarantus retroflexus* by the domestic fly and one beetle; *Lepidium Smithii* by small flies and many beetles of the genus *Altica; Angelica pyrenæa* by one beetle and two flies; three species of *Euphorbia* were visited only by flies; *Salsoda Soda* by *Syritta pipiens* and microscopic Diptera; among brownish flowers *Pelargonium triste* was visited by one fly; *Vincetoxicum purpurascens* only by *Musca domestica;* the brown-violet flowers of *Veratrum nigrum* and the reddish flowers of *Neottia Nidus-avis* only by flies. According to this list, in a few instances greenish flowers secreting nectar very freely are visited by a large number of insects, but the majority of the species are evidently not well adapted to entomophily.

[4] Root, A. I., "The ABC of Bee Culture," p. 38, 1903.

This conclusion is sustained by an examination of the green flowers of eastern North America. In the territory east of the 102d meridian and northward of North Carolina and Tennessee there are 1,244 green or dull colored flowers, of which 1,021 are anemophilous or hydrophilous, while 223 are entomophilous or autogamous. The wind-pollinated flowers are small and usually greenish, as in the 705 species of grasses and sedges, from which the inference is commonly drawn that anemophily and inconspicuousness are correlated. In the few exceptions (about 27 in the flora of the eastern states) where anemophilous flowers have brighter hues, as in the golden yellow aments of the yellow birch and the deep red panicles of the field sorrel (*Rumex Acetosella*), the colors of the small flowers are usually determined by the production of yellow or red pigments in great abundance by the vegetative organs—the entire plant of the field sorrel being sometimes red-colored.

Of the 223 green flowers, which are entomophilous or autogamous, many have no petals, as fifteen species of the Polygonaceæ and eight species belonging to the Caryophyllaceæ, also in several Rosaceæ, in *Acer saccharinum* and *Didiplis diandra*. Many are self-fertilized, as *Triglochin* and *Scheuchzeria*, and the orchids *Habenaria hyperborea* and *Epipactis viridiflora*, and the small green flowers of *Lechea* and *Penthorum sedoides*. Some have the petals caducous and depend upon their scent to attract insects, as the Vitaceæ. Many are visited chiefly by flies and the smaller bees, as various Melanthaceæ, the Smilaceæ, and the green flowers of the Asclepiadaceæ. A few species secrete nectar freely and attract numerous visitors, as the rock maple, basswood and the dark green pistillate flowers of *Rhus typhina*. Large green flowers, which are fragrant, nocturnal and are pollinated by moths, are found in exotic Solanaceæ and Orchidaceæ. It is obvious that bright coloration is less important to moth flowers than a strong scent, since red and blue

shades are invisible at night. But as a whole green flowers are small or even minute and attract few insects.[5]

Since in Europe and America, where the insect fauna is rich both in species and individuals and the flora displays a great variety of brilliant hues and delicate shades, dull-colored flowers are not well adapted to entomophily, it may be inferred that in a country where the flowers are largely greenish there would be a scarcity of anthophilous insects. According to A. R. Wallace and G. M. Thomson this condition is partially realized in the Islands of New Zealand. Wallace says:

> In New Zealand where insects are strikingly deficient in variety the flora is almost as strikingly deficient in gayly colored blossoms. Of course there are some exceptions, but, as a whole, green, inconspicuous and imperfect flowers prevail to an extent not equalled in any other part of the globe, and affording a marvellous contrast to the general brilliancy of Australian flowers, combined with the abundance and variety of its insect life. We must remember, too, that the few gay or conspicuous flowering-plants possessed by New Zealand are almost all of Australian, South American, or European genera. . . . After the preceding paragraphs were written, it occurred to me that, if this reasoning were correct, New Zealand plants ought to be also deficient in scented flowers, because it is part of the same theory that the odors of flowers have, like their colors, been developed to attract the insects required to aid in their fertilization. I therefore at once applied to my friend Dr. Hooker, as the highest authority on New Zealand botany; simply asking whether there was any such observed deficiency. His reply was, New Zealand plants are remarkably scentless.

After quoting the above passage, G. M. Thomson, who resided in New Zealand and made a special study of its floroecology adds:

> It is impossible to differ from this reasoning in toto, because the statements and facts on which it is founded are to a great extent correct, though in the light of more recent knowledge they require considerable modification.[6]

Bees and butterflies, according to Thomson, are comparatively rare; while Diptera, of which it is estimated

[5] Lovell, John H., "The Colors of Northern Gamopetalous Flowers," AMER. NAT., 37, 365–384 and 443–479.

[6] Thomson, G. M., "On the Fertilization of New Zealand Flowering Plants," *Trans. Proc. N. Z. Inst.*, 13, 241–288, 1880; Wallace, A. R., "The Geographical Distribution of Animals," 1, 457–464.

there may be a thousand species, are here the chief agents in the pollination of flowers, whereas in America and a large part of Europe they occupy the second place and in the Alps the third place. Neither honey-bees nor bumblebees were found in New Zealand at the time of its discovery.

The phylogenetic history of green flowers likewise strongly supports the view that they are not well adapted to pollination by insects. In the opinion of many eminent botanists all greenish, inconspicuous flowers have been derived by retrogression from larger entomophilous ancestors. This theory has been very ably developed by Professor C. E. Bessey in his taxonomy of the Angiosperms, for which he suggests the restoration of the more appropriate name of Anthophyta. The buttercups (Ranales), the water plantains (Alismales) and roses (Rosales) are regarded as primitive and are placed at the beginning of the Anthophyta. The typical flower was entomophilous, of large size, and its organs were separate and spirally arranged. Engler's spiral series of Monocotyledons, which is composed of orders mostly devoid of a perianth, is derived from a liliaceous type; while the Apetalæ are treated as reduced forms and distributed among the petalous Dicotyledons.[7]

A similar view is adopted by Arber and Parkin in their discussion of the origin of Angiosperms. They reach the conclusion "that the Apetalous orders without perianth, such as the Piperales, Amentiferous families and Pandanales, can not be regarded as primitive Angiosperms, but have been derived from ancestors with a well-developed perianth. Entomophily . . . has supplied the 'motive force,' which not only called the Angiosperms into existence, but laid the foundation of their future prosperity."[8] Even if Engler's system of classification

[7] Bessey, Charles E., "The Phylogeny and Taxonomy of Angiosperms," *Bot. Gaz.*, 24, 145–178, 1897; "A Synopsis of Plant Phyla," *University Studies*, 7, 275–373, 1907; "The Phyletic Idea in Taxonomy," *Science*, 29, 91–100, 1909, etc.

[8] Arber, E. A. Newell, and Parkin, John, "On the Origin of Angiosperms," *Journal of Linnean Society* (Botany), 38, 29–80, 1907.

is accepted and the apetalous orders be regarded as primitive it does not support the thesis that small, green flowers are at no disadvantage in attracting insects because of their inconspicuousness. That reduction and change from entomophily and conspicuousness to anemophily and inconspicuousness has occurred repeatedly in widely separated families is not questioned by any ecologist or taxonomist. This is illustrated by the genera *Artemisia, Ambrosia,* etc., among the Compositæ; in *Ricinus* of the Euphorbiaceæ; probably also in the family Juncaceæ, and in species of *Thalictrum, Fraxinus, Sanguisorba* and *Poterium.* The evidence supplied by the phylogeny of green flowers is wholly in favor of the value of color contrast for gaining the attention of insects.

Approaching the problem from another direction, the Rev. George Henslow in his work on the self-fertilization of plants finds that "inconspicuous flowers are almost invariably self-fertilizing, or else inconspicuous."

There are several reasons why inconspicuous flowers are not likely to be intercrossed by insects: (1) Their unattractiveness; (2) the absence of honey-secreting organs; (3) the want of scent; (4) they frequently do not expand, or at most remain half open, especially in cold or inclement weather, while perfectly cleistogamic flowers are, of course, never open; (5) their structure sometimes would seem absolutely to prevent the ingress of insects (such appears to be the case with *Polygonum Convolvulus* and *P. hydropiper,* the flowers of which seem to be always closed, and with many others.

He regards existing inconspicuous forms not as primitive, but as derived from conspicuous progenitors, which in turn owed their origin to the selective influence of insects.[9]

It has been shown that Plateau's conclusion is not sustained either by the phylogeny and distribution or by the ecology and manner of fertilization of inconspicuous flowers, which have almost universally been compelled to adopt anemophily or autogamy. In the few exceptional cases there are present other allurements, as odor and nectar, which sooner or later attract insects; but this

[9] Henslow, George, "On the Self-fertilization of Plants," *Trans. Linn. Soc.* (Botany), Ser. 21, 317–398, 1880.

does not prove that cœteris paribus color contrast is not an advantage. No assertion is made that bees have an antipathy to green, only that flowers of this hue are not as readily seen amidst the foliage. Very likely a green flower opposed to red or yellow leaves would attract the attention of insects as readily as the reverse contrast. Following the example of Plateau, I have included the species of *Tilia* among greenish flowers, but it is doubtful whether the inflorescence of the American basswood should be considered as inconspicuous. The flowers are of medium size, sweet scented, produced in vast quantities, and are described by a disinterested observer as "yellow and rather pretty." The nectar is so copious that a single hive of bees has obtained 66 pounds in three days, and its odor is so strongly aromatic that it can be perceived throughout an entire apiary.[10] It may, however, serve as an example of an exceptional species. The importance of scent as an attractive factor was, of course, recognized by Müller, but green flowers are usually odorless, as pointed out by both Hooker and Henslow.[11]

As additional evidence that insects will visit green flowers Plateau describes how he placed honey on seventeen anemophilous flowers, as grasses, sedges, rushes and on species of *Rumex* and *Chenopodium,* and observed visits of honey-bees, flies and a few other insects.[12] He also fashioned crude imitations of flowers from the living leaves of the red currant (*Ribes rubrum*) and the sycamore (*Acer Pseudo-Plantanus*), in which he put

[10] Root, A. I., "The A B C of Bee Culture," p. 397, 1903. A single colony of bees belonging to the late Dr. Gallup, of Orleans, Iowa, once gathered 600 pounds of basswood honey in thirty days. Doolittle, G. M., "Honey from Basswood," *Gleanings in Bee Culture*, 36, 23, 1908.

[11] A nectariferous flower may be both green and scentless and yet be found by bees. According to Fritz Müller the flowers of a species of Trianosperma in South Brazil are visited very abundantly all day long by *Apis mellifica* and species of *Melipona,* although they are scentless, greenish, quite inconspicuous, and to a great extent hidden by the leaves. "The Fertilization of Flowers," p. 270.

[12] Plateau, F., "Comment les fleurs attirent les insectes," *Bull. Acad. roy. Belgique,* 4me partie, 3me série, 34, 602–612, 1897.

honey, and they attracted bees and flies.[13] Attention is also called to the secretion of nectar by extra-floral nectaries upon the petioles of *Prunus*, the stipules of *Vicia* and the leaves of various trees. These sources of sweet secretions are frequently visited by Hymenoptera and other insects, as well as over-ripe or partially decayed fruit.[14]

A more interesting example than any given by Plateau of the secretion of nectar by extra-floral nectaries is furnished by the American cotton plant. Besides the nectar glands within the flowers there is a small gland on the center rib on the under side of each leaf, which at times secretes nectar very freely. When the atmospheric conditions are right, says Mr. J. D. Yancey in a recent number of *Gleanings in Bee Culture*, drops of nectar will collect on these leaf glands so large that they may be readily tasted; and a bee has to visit only a very few to obtain a load.

> At such times they neglect the blossoms entirely, and the honey comes in with a considerable rush. I could not tell that this honey was any different in either color or flavor from that gathered from the blossoms.

No other plant in this country besides cotton is known to me which has extra-floral nectaries, which are of value as a source of honey; but in favorable years there occurs on a scale of enormous magnitude an illustration that honey-bees will readily learn to gather sweet liquids from green leaves. In many parts of Europe and America Aphididæ, or plant-lice, and scale insects (*Lecanium*)[15] excrete a sweet substance called honey-dew in such large quantities that not only the leaves of the trees, but even the grass and the sidewalks, are coated with it as with a varnish. Honey-dew is attractive to many insects, as bees, ants, wasps and flies. In California it is sometimes

[15] In Hawaii enormous quantities of honey-dew are produced by a leaf-hopper. Phillips, E. F., "The Source of Honey-dew," *Gleanings in Bee Culture*, 38, 177, 1910.

[13] *Loc. cit.*, 5me partie, p. 868.

[14] *Loc. cit.*, 4me partie, p. 604.

so abundant that bees gather large stores of it, and Professor Cook says that he has sold it by the barrel.[16] From Sevensville, Montana, a correspondent of *Gleanings in Bee Culture* wrote a few years ago that the honey-dew had been in a continuous flow throughout the whole season, and dripped on the sidewalk every night in large quantities. Another bee-keeper at Dupont, Indiana, states that, in 1884, his bees gathered about two tons of honey-dew from the leaves of the oak, hickory, beech and wild grape. But the year, 1909, was in the opinion of well-informed apiarists the greatest year for honey-dew ever known in America. Bee-keepers everywhere reported a scarcity of white clover and basswood honey and that the bees were storing honey-dew. Professor Surface says:

> I have never known a year in all my studies of entomology, and in a correspondence of thousands of persons each month, during which plant-lice, or aphids, have been so abundant as they have this year (1909), and consequently the honey-dew was likewise unusually abundant.[17]

Many tons of this sweet excretion were consumed by the bees during the following winter.

I have dwelt at some length on the collection of honey-dew in order to establish beyond any question, not only that domestic bees would, but that they do gather large supplies of sweet substances from green leaves. If additional evidence could strengthen this statement it is furnished by every apiary, where bees frequently may be seen feeding on materials of every hue, or entering dark supers, hive-bodies, or boxes, through narrow crevices or small apertures no larger than a bee's body in search of honey. Honey-bees require a great amount of stores and it would be greatly to their disadvantage, if their actions were dominated by bright coloration to such an extent that they were prevented from obtaining food supplies

[16] Root, A. I., and Root, E. R., "The A B C and X Y Z of Bee Culture," p. 273, 1910.

[17] Surface, H. A., "Sources of Honey-dew," *Gleanings in Bee Culture*, 37, 623, 1909.

from every available source. It is, then, freely admitted that bees will collect sweet liquids, after they have once been found, from green or dull-colored surfaces; but this is very far from proving that bright coloring is not an advantage to flowers, and it is astonishing that such a claim based on the above facts should ever have been made.

Knuth in reviewing the observations of Plateau on greenish and brownish flowers very properly raises the objection that "Plateau has not compared the frequency of insect visits to inconspicuous and conspicuous flowers of the same size, and it is only experiments of this kind which can help to settle the point at issue."[18] This omission is fatal to Plateau's argument, and it is difficult to understand why control experiments were not employed. It is the object of the present paper to present the results of a long series of experiments, in which honey-bees under similar conditions were given the choice between a conspicuous and an inconspicuous object.

As a preliminary inquiry it is of interest to determine whether plants with diœcious inflorescence afford any assistance in deciding this question. As is generally known, the staminate flowers of entomophilous and sometimes of anemophilous diclinic species are more conspicuous than the pistillate. This is well shown by the genus *Salix*. Willow branches bearing staminate aments are offered for sale in New England cities in early spring, and are used for decorative purposes in the churches of England on Palm Sunday. Careful observation and collection of the visitors of *Salix discolor* (the glaucous or pussy willow), the earliest species of *Salix* to bloom in this locality, shows that the number of insects attracted by the staminate aments is much greater than by the pistillate. The difference is, indeed, so marked as to be readily apparent to any one who will keep an individual shrub of each form under observation for a few hours.

[18] Knuth, Paul, "Handbuch der Blütenbiologie," 1, 394, 1898; "Handbook of Flower Pollination," translated by J. R. Ainsworth Davis, 1, 207, 1906.

Another common diœcious plant is *Rhus typhina* L. (*Rhus hirta* (L.) Sudw.).[19] The staminate flowers are in large white panicles. The thyrsoid, pistillate flower-clusters are dark green; but as they are terminal and borne well above the foliage they are visible at a long distance, *i. e.*, they have conspicuousness of position. Two large groups of this shrub, or small tree, one of which was pistillate and the other staminate, growing in an open woodland only a short distance apart, were selected for observation. During two collecting trips in July, 1909, I secured on the staminate blossoms 77 visitors, but only 6 on the pistillate. Of the visitors to the staminate flowers 63 were bees, 2 wasps, 2 flies, 8 beetles and 2 Hemiptera. Of the visitors to the pistillate flowers 4 were bees and 2 wasps. Even a brief inspection is sufficient to show that the staminate flowers are more attractive to insects than the pistillate.

The staminate inflorescence of *Salix discolor* and *Rhus typhina* is, then, undoubtedly more conspicuous and attractive to insects than the pistillate; but is this larger company of visitors due wholly to its brighter coloration? Evidently not. Great numbers of honey-bees and many species of *Andrena* frequent the staminate aments of *Salix* to procure pollen for brood-rearing. At least five species of *Andrena* are oligotropic visitors of this genus. Examination of the polleniferous scopa of the bees taken on the staminate flowers of *Rhus typhina* showed that they all contained pollen except nine specimens of *Prosopis* (*P. modesta* Say and *P. ziziæ* Rob.), a primitive genus, the species of which possess only feebly developed brushes on the posterior legs, which are not used for carrying pollen. Of the eight species of beetles taken on the staminate flowers microscopic examination showed an abundance of pollen on the mouth-parts of four. The other four beetles were of small size, and it was not definitely determined whether they or the other insects mentioned above were feeding on pollen or not. But this

[19] The flowers of *Rhus typhina* are given as polygamous in most plant manuals, but they are certainly diœcious, as is also stated by Müller.

was of little consequence, since the proof is ample that in the case of the staminate plants of *Rhus typhina,* as in *Salix,* the pollen is an important factor in attracting visitors.

It is commonly believed that insects are attracted first to the staminate flowers of entomophilous diœcious plants by their greater conspicuousness, from which subsequently they carry pollen to the pistillate flowers. For instance, Müller says of the diœcious flowers of *Asparagus officinalis:* "This instance confirms Sprengel's oft-repeated rule that the male flowers of diclinic plants are more conspicuous than the female, whence insects are likely to visit the two kinds of flowers in their proper sequence."[20] But it is clear that many insects never fly to the pistillate flowers, since if they did the number of visitors to the two kinds of flowers would be equal. This is especially true of female bees, which, having obtained their load of pollen, often return directly to the hive or nest. Of the four bees taken on the pistillate flowers of *Rhus typhina* only two had pollen on the scopa of the hind legs. After collecting the pollinators of this species for several seasons I think it probable that some of the species of bees taken on the staminate flowers are never, or very rarely, found on the pistillate blossoms.[21] Sprengel's rule must, therefore, be accepted with considerable reservation.

The observations on diœcious flowers not proving well adapted for the purpose intended, owing to the presence of pollen as an attractive factor, the following experiment was tried. The flowers of *Gerardia purpurea* have a rose-colored, campanulate corolla and a short bell-shaped calyx. The species is common in this locality and is sparingly visited by bumblebees. When a large bouquet of the flowers was placed in front of a hive of black bees, it received very little attention. Apparently they contained no nectar. I now placed in the throat of a

[20] Müller, H., "The Fertilization of Flowers," p. 549.

[21] This is also true of dichogamous flowers. Robertson, C., "Flowers and Insects," *Bot. Gaz.,* 27, 41, 1899.

large number of flowers a small drop of honey. From a number of other stems I removed all the corollas and all conspicuous buds, and between the green calyx teeth I also put a drop of honey. So abundant was the honey on the green calyces that it could be seen at a distance of four feet. I could detect no scent in the complete flowers; certainly they seem to possess none comparable with that of honey. The two clusters of plants, the one decorallated and the other with its flowers complete, were placed on opposite sides of a glass of water, which was set near the entrance of a hive of black bees. The bees immediately showed a decided preference for the flowers retaining their corollas, as many as five visiting them at one time; while there were no bees on the denuded flowers though they were on the side of the glass nearer the hive. Later the bees discovered, as was to be expected, the honey on the green calyces and removed it. It is evident that to place honey on small green flowers, as in the experiments of Plateau with grasses and sedges, and when it is finally found by insects to conclude that conspicuousness is not an advantage is unjustifiable. The bees gave a decided preference to the brighter-colored flowers, and the fact that they subsequently discovered and removed the honey from the green calyces furnishes no evidence whatever against the benefit of color contrast.

But a method of experimenting was wanted, which would permit of varying the conditions under which the conspicuous and inconspicuous objects were exposed, and of counting the number of visits to each. This was obtained in the following manner: A small number of honey-bees were trained to visit for honey an unpainted, dull-gray board raised upon a support two feet high. A short time before the honey, which was placed directly on the board, was wholly removed, a conspicuous and an inconspicuous object were placed at equal distances from the board and at a known distance from each other. As soon as the bees had consumed the honey they began describing a series of ever widening circles in search of a new supply until one or both of the above-mentioned

objects were discovered. The number of visits made to each in a given time was then counted, and served as a basis for estimating numerically the value of conspicuousness.

On October 1, 1909, a small number of bees were accustomed to visit the dull-gray board, on which there was a small quantity of honey. For convenience this board will be called the feeder. While the bees were busily at work, I put a blue slide (prepared by placing the floral leaves of the bee larkspur (*Delphinium elatum*) between two glass object slides, 3 × 1 in.), on the center of which there were a few drops of honey, on the grass of the lawn about three feet from the base of the feeder; and on a dandelion leaf three feet from the base of the feeder and five feet from the blue slide honey was also placed. As soon as the supply of honey on the feeder was exhausted the bees began circling in the air. In a few minutes one bee had found the blue slide, in ten minutes two bees, and in twenty-five minutes five bees; but none had found the honey on the dandelion leaf. I now placed beside the dandelion leaf an apple leaf with a comparatively large quantity of honey on it, and at the end of forty minutes one bee found it and a little later a second bee. I doubt if they would have found it then had they not for some time previously been flying low searching for honey in the grass, having from their previous experience with the blue slide learned to look for it there. In this experiment the advantage was clearly on the side of the conspicuous object. It would appear that if two flowers were blooming at some distance apart, the one bright colored and the other green, the former would be the more likely to be pollinated.

On October 3, at 12:33 P.M., I repeated this experiment. The blue slide, a dandelion leaf, both on the grass, and the base of the feeder formed the angles of an equilateral triangle, each side of which was three feet. Honey was placed on all three as before. Two minutes after the last drop of honey on the feeder had disappeared three Italian

bees found the blue slide. At 12:40 there were eight bees on the blue slide, but not one had found the honey on the dandelion leaf. Five minutes later there was one bee on the leaf.

If bees are guided by odor exclusively in their search for nectar, and contrast in color with green foliage is no advantage to flowers, then it would seem as though they should find a quantity of free honey as readily as when it is associated with bright coloration. About thirty Italian bees were accustomed to visit the gray-colored board, or feeder, which, as previously stated, rested upon a support two feet high. Six feet from this support and six feet apart, the three forming an equilateral triangle, were placed two poles each 4½ feet high. On top of one of the poles was placed a quantity of honey so large that it ran down on the side, and was visible at a distance of twenty feet. To the top of the other pole was attached a cluster of yellow "immortelles" (*Helichrysum bracteatum*) gathered many years ago, and which appeared to be absolutely devoid of scent. Each of the flowers was about 1¼ inches in diameter and the cluster was 3 inches long by three inches wide. At 11:10 A.M., the bees were permitted to consume all the honey on the feeding board. In three minutes there were three bees and one fly on the flowers, but no insects had found the free honey. In five minutes there were four bees and one fly on the flowers, and one bee on the free honey. At 11:20 the latter bee left for the hive and five minutes later returned; a second bee also alighted on the side of the pole and began sucking the honey which had run down from above; two flies, apparently house flies, also came. At the same time there were six Italian bees on the flowers. At 11:30 A. M., there were six Italian bees and one fly on the flowers, but only one bee on the free honey. The flowers not only attracted the bees earlier than the free honey, but three times as many of them.

I now transposed the poles. But to the top of the pole on which there had previously been the supply of free

honey I fastened a single yellow "immortelle" one inch across. The individual flower enjoyed the advantage of position since it stood where the cluster had been before. Honey was placed on all the flowers. At 11:50 A.M., there were nine Italian bees and a Syrphid fly on the cluster of flowers and three Italian bees and one fly on the single flower. The larger and more conspicuous object notwithstanding its changed position received the greater number of visitors.[22]

The following experiments were made in 1910, and only black bees were employed. As in the experiments of the preceding year, the bees were trained to visit the same dull gray board placed upon a support two feet high. On September 14, 1910, at 12:40 P.M., the bees were carrying away syrup of sugar from the feeder. Nine feet from its base I put out on the grass of the lawn a dried yellow flower of *Helichrysum bracteatum* 1¼ inches in diameter, containing a small quantity of honey. On the opposite side of the feeder at a distance of nine feet from its base I laid a Red Astrachan apple leaf, 2 inches long by 1½ wide, on the center of which there was an ample supply of honey. There were at least twenty-five bees on the board and later the number increased. At 12:55 they had wholly consumed the sugar syrup. At 1:07 a bee came to the flower, but left almost immediately. At 1:10 a second bee came to the flower, but soon left, and a few moments later a third visit was made in the same way. No bees had found the leaf. As the honey was excellent I could account for the brief stop made by the bees only on the ground that they were looking for sugar syrup. In the next experiment this was offered to them.

At 1:20 P.M., I again put sugar syrup on the feeder, and removed the flower and leaf from the grass. Another "immortelle" 1¼ ins. in diameter and another Red Astrachan apple leaf, 2 inches long by 1½ inches wide, were laid on the grass on directly opposite sides of the feeder,

[22] Cf. Müller's remarks on *Geranium, Epilobium, Polygonum* and the Alsineæ.

but six feet instead of nine feet away from its base. The leaf was on the same side as before, as was also the flower. Sugar syrup, which is odorless, was placed on each. At 1:30 the bees finished the syrup on the feeder. One bee flew almost immediately to the flower, but made a brief stay. At 1:34 a second bee came and sucked, and three minutes later a third bee came. No bees had found the leaf.

Sugar syrup was again put on the feeder, and the flower and leaf were moved three feet nearer its base, each now being distant three feet. At 1:47 the syrup on the feeder was all consumed, but even previously one bee had come to the flower. At 1:47 a bee flew over the leaf, but did not alight. At 1:50 three bees came to the flower, and a moment later a fourth, and afterwards two more. At 2 P.M., there were three bees on the flower, a fourth came a little later and then a fifth. No bees had visited the leaf.

Syrup of sugar was again placed on the feeder. At 2:5 P.M., I put out the yellow flower and apple leaf used in the first experiment. On these, it will be remembered, honey had been placed. They were laid on the grass on opposite sides of the feeder, each three feet distant from its base. At 2:10 the sugar syrup on the feeder was all removed. A bee soon came to the flower, but did not stop, a second bee came and sucked, a third bee came, but did not stop, several bees came but did not stop; but at 2:13 there were three bees sucking honey on the flower. A bee flew slowly over the leaf I thought it would certainly be attracted by the scent of the honey, but this was not the case. The experiment was continued a little longer and one or two more visits were made to the flower, but none to the leaf.

The results obtained in the four preceding experiments are deserving of careful attention. While the yellow flower containing honey and the one containing scentless sugar syrup were visited many times by bees, the leaves remained wholly unvisited, though the supply of syrup

or honey on them was plainly visible at a considerable distance. According to the reiterated statement of Plateau all flowers might be as green as their leaves without their pollination being compromised, and color and form are of little consequence in comparison with odor. But the experiments showed that color contrast is of great value, and in these particular experiments indispensable. If the leaves provided with an ample supply of honey or syrup could not obtain a single visit under the conditions described, where a large number of bees were brought into their immediate vicinity, how little chance there would be for an isolated plant with small green flowers growing in a secluded location attracting visitors! But a bright-colored flower in the same locality would be much more likely to gain the attention of pollinating insects.

On September 20, 1910, at 2:15 P.M., numerous black bees were coming to the feeder for honey. At a distance of three feet away I laid on the grass a bright yellow flower of golden glow (*Rudbeckia laciniata*) two inches in diameter. On the opposite side of the feeder three feet from its base, I laid the end of a spike of *Amarantus retroflexus* about three inches long. The small, pale green flowers are thickly crowded in panicled spikes. An ample supply of honey was placed on both. In the course of fifteen minutes there were 18 visits to the flower of golden glow and only 8 to the *Amarantus* cluster. If a bee flew to either object, but did not alight because of the large number of bees already there, this was counted as a visit.

The bees were again accustomed to visit the feeder. In the preceding experiment one of the objects had been placed on the east side of the feeder and the other on the west. Both the flower of the golden glow and the spike of *Amarantus* were now laid side by side on the grass in the sunshine three feet to the north of the feeder. There was honey on both. In less than ten minutes there were fifteen visits to the golden glow and only three to the

spike of *Amarantus*. At one time there were five bees on the golden glow and only two on the spike of *Amarantus*.

At 2:45 P.M., I repeated the preceding experiment, but I placed the flower of the golden glow and the spike of *Amarantus* on the south side of the feeder three feet from its base, but only three inches apart. Honey was put on both at the beginning of the experiment. In ten minutes there were 18 visits to the golden glow and 5 to the green spike of *Amarantus*. At one time there were four bees on the flower of golden glow, but only one on the spike of *Amarantus*. It often happens when a bee comes to a flower on which one or more bees are already at work that they will all fly up in the air and then all or in part settle down again. Such flights were not counted. Frequently a bee flew directly to the golden glow as though it had been seen from a distance.

It will be remembered that Plateau put honey on the green inflorescence of several species of *Chenopodium*, besides other anemophilous flowers, and when it was found by insects reasserted his oft-repeated conclusion that winged pollinators are guided to flowers almost exclusively by odor and that color contrast is of little value. Plateau employed no control experiments, but it appears from the experiments just described that though the odor of the honey drew insects to the green inflorescence, nevertheless it was at a disadvantage because of the absence of bright coloration.

In several of the experiments of 1909 a blue slide was used, prepared by placing the leaves of the perianth of the bee larkspur (*Delphinium elatum*) between two glass object slides tied firmly together with black silk. It might perhaps be objected that the scent of these floral leaves would escape through the narrow crack between the two glass slides. While I think this improbable, and that in any event it would be so slight as to bear no comparison with that of the honey placed upon the upper glass slip and, therefore, would exert no influence on the

behavior of the bees, still it seemed desirable to test the matter. For this purpose the following experiment, closing the series of 1910, was performed on September 23. A blue slide was prepared as described and the edges were sealed with several applications of gold size, the odor of which is no doubt unpleasant to bees. The blue slide, a dandelion leaf, and the base of the feeder formed the angles of an equilateral triangle, each side being three feet in length. As the weather was becoming colder the bees were not flying freely. An ample supply of honey was put on the blue slide and the leaf, which were laid on the grass of the lawn at 9 P.M. At 9:20 the honey on the feeder was entirely consumed. Presently a bee hovered over the blue slide, but did not alight. Another bee hovered over the blue slide for a long time and finally alighted. A second, third and fourth visit was made by bees at intervals. At 9:40 I discontinued the experiment. No attention had been paid to the honey on the leaf, though in the sunlight it could be seen for a long distance. The hesitation of the bees at first may have been caused by the repellent odor of the gold size. Beekeepers never paint their hives inside, as the scent of paint is believed to be disliked by bees. The blue slide and the leaf were left in position and when twenty minutes later I examined them again all of the honey had been removed from the slide, but that on the leaf appeared to be untouched. Evidently the only factors which had influenced the bees in the previous experiments were the honey and the color.

Of the series of experiments performed in 1911 only three will be described. A few observations were thought desirable in which one or two bees were employed instead of a larger number, in order that the behavior of an individual bee might be followed when given the choice between a conspicuous and an inconspicuous object. A few bees were accustomed to visit a glass slide for honey. While they were absent at the hive, the slide was removed and a large rhubarb leaf was laid in its place.

About two inches from the base of the leaf there was put a quantity of amber-colored honey sufficiently large to form an oval mass, which could be seen in the shade at a distance of twenty feet. Twelve inches from the honey and a few inches from the apex of the rhubarb leaf there was placed a bright red flower of the Zanzibar balsam (*Impatiens sultani*), an inch in diameter, on which there was a small amount of honey.

A bee returning from the hive went directly to the red flower, where it took up its load and flew away.

A bee came to the red flower. Two more bees came and were impounded. The first bee left for the hive.

A bee returned to the flower. A second bee came, both flew up in the air, and one of them went to the mass of honey but soon returned to the flower. The first bee left for the hive. I attempted to impound the second, but it escaped.

A bee came to the flower, and after five minutes returned to the hive.

The bee returned to the flower. A second bee came, and hovered in the air for some time, but finally settled by the bee on the flower. Both bees left for the hive. Both bees returned to the flower, and when they again left I discontinued the experiment. The rhubarb leaf was removed and the bees were given honey on a glass slide.

It seems impossible to explain the behavior of the bees in this experiment on the supposition that they were guided chiefly by odor. In view of the large quantity of honey and its easy accessibility there would have been no occasion for surprise had the bees given it much greater attention.

After carefully removing the honey from the rhubarb leaf I placed near its apical end four flowers of the Zanzibar balsam, forming a bright red square. On one petal of each flower there was a small drop of honey. Ten inches away near the base of the rhubarb leaf I put a single petal of a balsam flower on which there was a large drop of honey. While both bees were away I removed the

glass slide and substituted the rhubarb leaf, reversing its position, however, so that the small object was where the larger had been before.

Both bees returned to the cluster of four balsam flowers. One of them presently flew over to the single petal, but soon returned to the cluster; later it again went to the petal and again returned. Both bees left for the hive.

A bee returned to the cluster, did not alight, but flew over to the petal and sucked. When the second bee returned it disturbed the bee on the red petal, and both went to the cluster. One of the bees left for the hive.

A bee came from the hive to the cluster. One of the bees then flew over to the petal but did not alight, returned to the cluster. Both bees left for the hive.

Both bees returned to the cluster. One of them left for the hive and on its return went to the petal. The bee on the cluster left for the hive.

While the cluster of four red flowers received the greater number of visits, as would be expected, more attention was given to the drop of honey associated with a red petal than was received by the larger oval mass of honey alone in the preceding experiment.

In a series of interesting experiments with cotton flowers where the visitors were chiefly a species of *Melissodes* (*M. bimaculata*), recently described by Allard, it was observed that when a flower was partially screened by leaves the attention it received decreased; and when the petals were masked on both sides with sections of green leaves the flower was ignored entirely.[23] This result was confirmed by the following experiment. On a cloudy, windy day while a number of black bees were visiting the feeder for honey, I placed on the grass two red flowers of the Zanzibar balsam; each was five feet from the base of the feeder and their distance apart was

[23] Allard, H. A., "Some Experimental Observations concerning the behavior of Various Bees in their Visits to Cotton Blossoms," AMER. NAT., 45, 615 and 672, 1911.

two feet. There was a small quantity of honey on both. One of these blossoms I screened with dandelion leaves on the side toward the feeder, but it was visible in every other direction. Some time after the honey was all consumed on the feeder two bees flew over the unconcealed flower but did not alight. A wasp (*Vespula victua* Sauss.)[24] found it and at the end of half an hour it was visited by a bee. The partially concealed flower received no attention. During this experiment the bees seldom inspected objects on the lawn though they frequently flew to where I was sitting, ten feet away.

The conclusion derived from a study of the phylogeny, ecology, distribution and fertilization of green flowers that they are at a disadvantage in attracting insects be cause of their color was fully sustained by a long series of experiments, in which honey-bees were given the choice between a green and a bright colored object placed on a green background, or between a conspicuous and an inconspicuous object. In the experiments described both black and Italian bees were employed, the number of which varied from one to fifty. The observations extended over portions of three seasons. Conspicuous and inconspicuous objects were in some instances placed diametrically opposite to each other at varying distances, in other cases side by side or a few feet apart. In six experiments there were no visits to the inconspicuous object; while in the other experiments the number of visits to the conspicuous object was much larger than to the inconspicuous object, usually twice or three times as large. The theory that bees in gathering nectar are influenced only by the olfactory sense and not by color or form does not afford a satisfactory explanation of the facts presented. If, however, bees are guided by the sense of vision as well as by that of smell, then their relations both past and present to green flowers are not difficult to understand. To reject a natural and wholly

[24] For the determination of this species I am indebted to Mr. S. A. Rohwer.

satisfactory explanation of their behavior in favor of an improbable hypothesis has the appearance of shunning the truth in a vain search for novelty.

Conclusions

Green flowers are not well adapted to entomophily, and many species, possibly all, have been derived by retrogression and degeneration from larger more highly developed entomophilous forms. They are usually small, or even minute, and are often incomplete, while anemophily and autogamy prevail. Entomophilous green flowers are as a whole sparingly visited by insects belonging to the less specialized families, and as a rule retain the power of self-fertilization.

The fact that insects have been observed feeding on over-ripe or decaying fruit, or the glandular secretions of the vegetative organs of plants, or the excretions of Aphididæ on foliage, or greenish or brownish flowers, or dull-colored receptacles which have contained sugar or sweet liquids, affords no evidence that conspicuousness is not an advantage to entomophilous flowers. Any surface, whether it is bright or dull-colored, on which there is nectar or honey, will be freely visited by bees for stores after these liquids have once been discovered; but they will not be discovered as quickly on a surface which does not contrast in hue with its surroundings as on one which does so contrast.

The experiments and observations of Plateau on green or greenish flowers in the absence of control or comparative observations are fallacious, as pointed out by Knuth, and do not prove that "all flowers might be as green as their leaves without their pollination being compromised."

When honey-bees are given the choice between a conspicuous and an inconspicuous object under similar conditions, they exhibit a preference for the former. This preference is sufficiently marked to account for the development of color contrast in flowers.

SHORTER ARTICLES AND DISCUSSION

IS THE CHANGE IN THE SEX-RATIO OF THE FROG THAT IS AFFECTED BY EXTERNAL AGENTS, DUE TO PARTIAL FERTILIZATION?

In a review in this journal (XLV, 1911) of certain experiments by Kuschakewitsch[1] on frogs' eggs in which by delaying fertilization for 89 hours he obtained 100 per cent. of males, I pointed out that unless more than half of the eggs were fertilized the *interpretation* of the 100 per cent. ratio might be misleading. For should the delay act more injuriously on one kind of egg than on the other, assuming two kinds to exist, the result might mean only selective destruction by an external agent rather than a change in sex of the eggs. I found no explicit statement in the section of Kuschakewitsch's paper dealing with these results to show whether or not all of the eggs had been fertilized, but in a recent rejoinder[2] to my review Kuschakewitsch points out that he had stated that practically all of the eggs ("so gut wie alle Eier") were fertilized and developed. This information is given in an *appendix* which I had overlooked. His statement completely sets aside the possibility of the suggestions that I made, but leaves the explanation of his results as obscure as before.

The details of the principal experiment and of some of the others are of interest. A pair of copulating frogs were caught at 12:00, midday, May 31. The female began to lay at once. At 6:00 P.M. the male was removed. On the 4th of June at 8:00 P.M. the eggs that had remained in the uterus of the female were artificially fertilized. They are recorded as 89 hours old at this time. Practically all segmented, and only 5 died at the gastrulation stage. From this lot, 434 eggs hatched. Only 12 deaths occurred later. Three hundred of the tad-poles were examined at the time of or after metamorphosis. Of these, 299 were males, and one was a bilateral hermaphrodite.

There can be little doubt, therefore, that, in some way, delay in fertilization has caused practically all the eggs to produce males; and the evidence is the clearer since the eggs fresh laid, fertilized by the same male, produced 55 males and 53 females.

It may seem futile, therefore, to attempt to explain this result in any other way than as the result of the action of the environment on the sex of the egg. But how has the environment

[1] *Festschrift*, R. Hertwig, Bd. II, 1910.

[2] *Anatom. Anzeiger*, 1911.

acted? The evidence that sex is regulated by an internal mechanism has become so strong in recent years that until the action of the environment is made clear one may well hesitate to accept the case as showing that sex is actually changed or produced by an external agent. Curiously enough, every one seems to have overlooked still another possibility that may solve the difficulty. The delay in the fertilization may cause the polar spindle to stick to the surface of the egg so that it fails later to take part in the development, in which case the sperm nucleus alone would produce the nuclei of the embryo. Or, on the other hand, the delay may cause the early stages in the formation of the female pronucleus to progress so far that after fertilization the sperm nucleus may be excluded in part or entirely from the development. In either case the presence of a single nucleus would be expected to give rise to a male. It is significant in this connection that the changes described by King that affect the sex-ratio of the frogs' eggs produce a higher percentage of males.

There is another curious fact in relation to sex-determination in the frog. Pflüger described a high percentage of hermaphrodites amongst the tadpoles. Kuschakewitsch has given a detailed account of the development of the hermaphroditic glands. Most or all of these organs are later transformed into testis. In general it may be said that eggs from a pair give either equal numbers of males and females; or a mixture of males, females, and hermaphrodites; or all hermaphrodites (potentially males). It is possible that the pseudo-hermaphroditic condition may be connected with the failure of one of the two pronuclei to take part in the development.

If the explanation that I have suggested is correct we might expect to find evidence in its support from the number of chromosomes in the tadpoles that develop from these late fertilized eggs. This would be expected if it is the male pronucleus that gives rise to the nuclei of the embryo. But if it is the female pronucleus that is responsible for the result, the number of chromosomes in the cells of the embryo might be haploid or diploid depending on whether the second polar body was, or was not given off. At any rate, this suggestion should be put to the test of observation before we conclude that sex may be determined by external agents. If the view here suggested prove true, sex is still determined by an internal factor in the same sense that the sex of the bee's egg is determined by the presence of one or of two pronuclei.

T. H. MORGAN

NOTES AND LITERATURE

HEREDITY

A SUBJECT of vital importance to the theory of heredity is the behavior of the chromosomes during the life history of the cell, and especially during the process of cell division. This subject has received an enormous amount of attention from investigators but there is far from unanimity amongst cytologists as to the actual phenomena of cell division, not to mention the significance of these phenomena. Realizing that the heterotypic division in gametogenesis is the critical point in the life history of the organism, so far as the theory of heredity is concerned, attention has been concentrated very largely on this division. This is in some respects unfortunate. A good many investigators who have studied this division have attempted to interpret the phenomena observed without full knowledge of the behavior of the chromatic elements in ordinary somatic divisions, and have attributed to phenomena observed in the heterotypic division very special meaning for inheritance, when these same phenomena are regular occurrences in all divisions, and hence are to be interpreted in their relation to growth rather than to reproduction.

The writer is not a cytologist, and realizes fully that his opinions on cytological questions will not be regarded seriously, especially by those who have worked at problems of this character until they have gotten fixed in mind certain theories as to the meaning of the phenomena observed. Nevertheless, he has given careful attention to published results of investigations of this character, and has been driven by study of these results to a particular interpretation of the principal phenomena reported. It has seemed to me for some years that the double spireme so often reported in the heterotypic division, and so often interpreted as a conjugation of homologous chromatin elements, although this double spireme occurs in somatic divisions, apparently quite as generally as in the heterotypic, is nothing more than the expression of a division of chromosomes which really occurs at least as early as the resting stage following the previous nuclear division. It appears that this division may begin at an even earlier period.

Fraser and Snell, in their paper on "The Vegetative Divisions in *Vicia faba*,"[1] present important evidence on this point. They show very clearly that the division begins in the teleophase of the previous division. This beginning of chromosome division in teleophase had earlier been noticed by Gregoire, and by Stromp, as pointed out by Fraser and Snell, but these earlier investigators had not perceived the meaning of this phenomenon. Fraser and Snell were able to follow the life history of the chromosomes through their complete history from one teleophase to the next (in root tips and in other somatic parts, as well as in the gametophyte stages), and they show clearly that the double nature of the spireme is due to splitting which begins as the daughter chromosomes congregate at the poles in the teleophase of the previous nuclear division.

That this double spireme is not due to the approximation of two elements, one representing maternal and the other paternal chromatin, is further shown by the fact that in the pollen cell, where the chromosome number is haploid, and hence where there can be no question of union of elements from the two parents, exactly the same phenomena occur.

Another very interesting fact shown by these investigations (of Fraser and Snell) is that some of the elements which behave as single chromosomes, so far as their distribution on the spindle and to the poles is concerned, are made up of segments united end to end, as if two or more small chromosomes were united more or less closely into a larger one. The authors point out the possible significance of this fact for Mendelian coupling, and suggest that it may also be of significance in connection with the fact that in some species more Mendelian factors have been observed than there appear to be chromosomes.

East and Hayes have recently published the results of extended investigations on inheritance in maize.[2] After discussing the taxonomy of the group and pointing out the adaptability of maize to genetic investigations (or the lack of such adaptability), the authors give an excellent résumé of former investigations with this interesting group of plants.

A brief account of their results follows. Amongst endosperm characters they found that starchiness (*S*) is dominant to its

[1] *Ann. of Bot.*, 1911, pp. 845–856.

[2] E. M. East and H. K. Hayes: Bul. No. 167, Conn. Exp. Sta.—"Inheritance in Maize."

absence, non-starchiness (*s*). When *S* came from the ♂ parent zenia appeared in all cases. All F_1 seeds showing zenia proved to be heterozygous. No extracted recessives of the F_2 generation ever proved to be heterozygous. "From this one may conclude that the second male nucleus that fertilizes the endosperm nucleus always bears the same characters as the first male nucleus that fertilizes the embryo nucleus, or egg." A few seeds, all heterozygous, were part starchy and part not; *i. e.*, one side was starchy. The authors consider that this confirms Correns's view that, in such cases, the second male nucleus did not fuse with the endosperm nucleus but that each developed separately.

One semi-starchy ear occurred, grown from a non-starchy seed. The authors suggest two possible causes for this phenomenon. Either there is an incomplete segregation, resulting in contamination of a gene by its allelomorph, this contamination, by selection, being capable of accumulative effects, or the semi-starchy ear arose as the result of a progressive variation. They point out that the infrequency of this phenomenon is an argument against the theory of partial or incomplete segregation, and incline to the idea that it is a case of progressive variation. The data presented certainly favor this interpretation of the case.

YELLOW AND NON-YELLOW ENDOSPERM

Two independent factors for yellow were found, each capable of producing yellow endosperm. The colors produced by these two factors appear to be the same. The pigments occur in rhombic plates, and are insoluble in water, but soluble in ether, chloroform, etc. They appear to be related to the anthochlorins.

Some crosses between yellow and white gave the ratio 3:1, due to the presence of only one of the factors for yellow. Others gave the dihybrid ratio. Yellow appeared as xenia in the hybrid seeds (seeds which produced the F_1 plants).

Yellow was dominant, but imperfectly so under certain conditions, so that, in certain crosses in which the grains had soft starch at the tip the heterozygotes could be distinguished from the homozygotes. In other crosses yellow was completely dominant.

The same original ear of some of the parent stocks had some seeds containing both factors for yellow, while other seeds on the same ear had only one of these factors.

The two yellow factors together generally gave darker yellow seed than one factor alone.

Purple and Non-Purple Aleurone Cells

The experimental data relating to crosses between purple and non-purple races indicate two factors, *P* and *C*, which, when together, produce purple color in the aleurone layer. In some of the non-purples used one of these factors was missing, in others both. In certain combinations one of these factors alone produced faintly colored purple. In most of the crosses splashed purple occurred, part, but not all, of the heterozygotes having this peculiarity. It was not hereditary, but behaved in subsequent generations as pure purple.

In one family red aleurone occurred in F_2. It appeared to be due to the interaction of two factors *R* and *C*.

In one family of this cross (purple $\times$ non-purple) the F_2 generation gave purples, reds, and non-purples in the ratio 12 : 1 : 3. The actual numbers were 1,843 : 188 : 545. The ratio 12 : 1 : 3 did not occur in F_3, but instead one fifth of the ears bearing F_3 grains gave the ratio 9 : 3 : 4. Several possible hypotheses to explain these anomalous results are discussed and discarded, amongst them Bateson and Punnet's hypothesis of the formation of gametes in the ratio $7AB : 1aB : 1Ab : 7ab$. The data from F_3 agree best with the assumption that the constitution of the two parent types was *pcR* and *PCR* respectively, but the F_2 ratio is not explained by this hypothesis. The authors leave the question as to the real explanation an open one. The reds in this family were different in color from the reds of the family previously mentioned. Not only that, but all the F_2 reds found in this family proved to be homozygous, indicating that both parents possessed the factor *R*.

Other families of this cross gave results that indicated the presence in the non-purple parent of a factor which more or less completely inhibited the development of purple. Some nonconformable results were found, due probably to the presence of other factors, one at least of which appeared to modify purple by making it lighter.

In the above crosses xenia was found as follows: when nonstarchy races were fertilized by pollen from starchy races (no xenia appeared in the reciprocal cross); when non-yellow endosperm is crossed with yellow endosperm. In this case xenia always appeared when yellow was used as the male parent. It also appeared in the reciprocal cross when the grains of the female parent had extensive development of soft starchy endosperm at the end of the grain, as in these cases the heterozygous

yellow was lighter in color than the homozygous, and hence distinguishable from it. In crosses between yellow and non-yellow endosperm, when the non-yellow endosperm was entirely soft (not corneous), as in the so-called flour corns, xenia appeared in all cases, for reasons just stated. When the endosperm of the non-yellow parent was entirely corneous, as in the popcorns, xenia usually occurred only when yellow was used as the male parent, though in a few instances it was perceptible when the cross was made the other way. Xenia also occurred when purple or red aleurone was crossed with non-purple, or non-red, when the purple or red was used as the male. The only other case in which xenia was observed was in crosses between white and red (or purple) when the white (male) parent carried an inhibiting factor for purple and red. Sometimes the reciprocal cross shows xenia, since the inhibition of red or purple is not always complete. The following law regarding xenia is formulated by the authors: when two races differ in a single visible endosperm character in which dominance is complete, xenia occurs only when the dominant character is the male; when they differ in a single visible endosperm character in which dominance is incomplete or in two characters both of which are necessary for the development of the visible difference, xenia occurs when either parent is used as the male.

Correns's conclusion that where xenia occurs the seeds showing it are always hybrid is confirmed. This shows that Mendelian segregation must occur previous to the division of the pollen nucleus. The authors found no case in which a seed showing no xenia where it is to be expected proved to be a hybrid; *i. e.*, the hybrid in which xenia is to be expected always showed xenia though like Webber and Correns they found seeds showing xenia on only one side. This is interpreted as the result of the independent development of the endosperm nucleus and the second male nucleus.

In crosses between podded maize (maize having each grain covered by husks) with common maize, the pod character proved to be a dominant Mendelian factor, which segregated perfectly in F_2.

Red sap colors appear in maize in the pericarp, the cob, the husks, the silks, the glumes, and in the anthers.

Red pericarp (*R*) without red on the cob or in the silk, crossed with white pericarp gave three reds to one white in F_2, the segregation being perfect.

An ear of corn was found where only white corn had been planted, one side of which produced grains with red pericarp, the other white or striped with red. The red here seemed to be due to the same factor as in the case just noticed (*R*). Red grains from this ear gave red and white ears in equal numbers. The white and striped grains gave white ears and ears with a few striped red seeds in equal numbers. A selfed red ear in this generation gave three reds to one white in the next. The original red and white (or striped) ear is accounted for as a somatic variation, part of the ear varying from white to red, the remainder from white to striped. In this family red cob is perfectly correlated with red pericarp.

Two other red pericarp colors, apparently independent of the above, were found. One is a dark red occurring in stripes which radiate from the point of the attachment of the silk to the grain. The other is a dirty red, more abundant at the base of the grain, and nearly wanting at the tip. It occurs in Palmer's Red Nosed Yellow variety. It is completely coupled with red silks.

Two other red pericarp factors were found. They are very similar, but not allelomorphic to each other. They give a rose red pericarp, but do not develop except in sunlight. They are barely perceptible on ears covered by heavy husks.

Red cob color proved to be dominant to white and the cross segregated in a 3:1 ratio. Red cob appears not to be correlated with any of the red pericarp colors.[3]

Red silk color presented some difficulties, and the data are not analyzed. In some instances a 3:1 F_2 ratio was obtained, in others a third type with red hairs on a greenish-white silk occurred, the F_2 numbers being 198 reds, 29 greenish-whites with red hairs, and 94 greenish-whites. Red silks may occur with no other red on the plant.

Red glumes were always accompanied by red in other parts of the plant, though in one race the only other red was in the silks.

The question whether all these reds are due to one or to different genes is discussed most interestingly (pp. 109–10), but the discussion is too long to quote here.

It is gratifying to note that these authors are not afraid to mention the chromosomes in connection with Mendelian factors. For several years an inexplicable obsession seems to have possessed biologists in this matter. Apparently every one regards

[3] But see reference to Emerson's results below.

it as highly probable that these cell organs are in some way responsible for Mendelian phenomena, yet a large number of biologists seem to be afraid to refer to them in this connection.

Crosses between flint and dent varieties indicated that the difference between these two classes relates to two factors in some case, especially when the dent parent has considerable corneous endosperm, and to two or more factors, especially when the dent parent has little corneous starch. This seems to be another case where several similar factors exist, as found in earlier investigations by East, by Nilsson-Ehle, and by Shull.

Crosses between races having different modal numbers of rows of grain on the cob indicate clearly that several similar factors are here concerned. The evidence that segregation occurs is, the authors believe, conclusive. The data could not be definitely analyzed because of the fluctuating variation of the various biotypes, and the small differences between adjacent biotypes. Height of stalk and length of ear behave similarly. Apparently size of grain does the same.

Irregular rows of grain occur both as fluctuations not inherited and as a hereditary characteristic. Should the percentage of irregular rows be higher than about 4 per cent. the authors think the irregularity is probably hereditary.

This paper closes with a discussion of the inheritance of various abnormalities found during the progress of the work.

The *Journal of Genetics* for August, 1911, contains some articles of unusual interest. R. N. Salaman presents the results of an investigation on the inheritance of the peculiar physiognomy known as the Jewish face. He shows that this is a simple Mendelian character. It is distinctly recessive to the ordinary European (Gentile) physiognomy, though the hybrids sometimes show traces of the Jewish face, especially late in life. On the other hand, this peculiarity is dominant over the Pseudo-Gentile face sometimes seen amongst the Jews; also to the Gentile physiognomy of the Moors and certain other Mediterranean peoples. We may explain this in terms of the presence-absence hypothesis by saying that the distinctive type of face seen amongst the Hebrews is due to the presence of a gene, while in the peoples of northern Europe there is a gene which inhibits the Jew face character. The data presented, while not extensive, seem to be quite conclusive that the character segregates as a so-called unit character. Salaman's paper is an exceedingly clear presentation of data, and is written in a style that is attractive and readable.

Bateson and Punnett[4] give the results of their study of the inheritance of the peculiar black pigmentation in the skin, periostium, and other tissues of the silky fowl. While some exceptions occur, the results on the whole are in agreement with the assumption of a pigment factor, *P*, and an inhibiting factor *I*, the latter exhibiting the phenomenon of spurious allelomorphism with the female sex factor. The authors suggest that the exceptions found may be due to failure of the repulsion supposed to exist normally between the female sex factor and the inhibiting factor, *I*.

While a large part of the work on which Mendel's principles of heredity depend has been done with pigments, very few investigations have been undertaken in order to determine the connection between the phenomena of inheritance of these pigments and the chemical reactions which underlie these phenomena. This is quite natural, since few of those who have conducted the investigations relating to Mendelian inheritance have had the training, and hence the opportunity, to study the chemical side of the question. Likewise, those relatively few individuals who have become well versed in the highly complex and difficult subject of physiological chemistry have seldom had any direct interest in the phenomena of inheritance. The wisdom of an endowment for an all-sided research of heredity such as the Carnegie Institution of Washington has provided at Cold Spring Harbor is manifest in the fact that Dr. Davenport has been able to institute research on both sides of the question. The results secured by Mr. Gortner in his study of the origin of melanin, and its relation to the phenomena of Mendelian inheritance will be eagerly read by students of Mendelism. In the May number of the *Journal of Biological Chemistry* Mr. Gortner gives some exceedingly interesting results of his work. He shows that the body filling of the meal worm (*Tenebrio moliter*) contains two oxidases, a laccase-like enzyme, and a powerful tyrosinase. Also that there is a chromogen present in the larva which, when acted upon by the tyrosinase, gives a series of colors ending in a black melanin-like body. Larvæ killed by ether developed pigment when left exposed to air, but when the air was excluded by carbonic acid or nitrogen no pigment developed. When the larvæ were heated (in water) sufficiently to destroy the activity of the tyrosinase but not that of the laccase, no pigment formed.

The oxidase was evidently present in relatively large amounts,

[4] *Jour. of Gen.*, Aug., 1911, pp. 185–203.

but the chromogen only in small quantities. The results indicate that the chromogen is formed slowly and used as formed.

In the September number of the same journal[5] the same author deals with the nature of dominant and recessive white. He shows that, in so far as the presence or absence of pigment is concerned, these two types of white, in certain mammals, are as indistinguishable to the chemist as they are to the breeder. He accepts the view that pigment is formed by the action of an oxidase on a chromogen, and points out that dominant white arises from the presence of a third body which prevents the reaction between the oxidase and the chromogen. This might occur in three ways: (1) the third substance, such as orcin, resorcin, phloroglucin, or other substances of similar nature, may act on the chromogen and thus prevent its oxidation; (2) it may itself be oxidized by the tyrosinase, thus preventing action on the chromogen; (3) it may act as a true anti-oxidase, and in some manner inhibit the action of tyrosinase.

The author gives abundant data to show that alternatives 1 and 2 are excluded in the cases with which he worked. Hence the action must be of the third type—an inhibitory action. He shows that dominant whites contain no pigment lacking in recessives. He also shows that "aromatic compounds which carry two hydroxyl groups in the meta position to each other are capable of inhibiting the action of tyrosinase on tyrosin." If, then, such a substance should occur in the animal body a dominant white would result. Recessive white is presumably due to the absence of either the chromogen or the oxidase, while at the same time no inhibiting factor is present. From this it would appear that an albino might be dominant to color if it carried the inhibiting factor, yet it would require considerable work to distinguish between such an albino and a true dominant white, *i. e.*, by its genetic behavior.

To explain the occurrence of recessive whites which are not albinic, and which are dominant in some crosses, we have the following considerations. Orcin inhibits the action of tyrosinase on tyrosine, but is itself oxidized by a specific enzyme. If we represent tyrosine by *C*, tyrosinase by *T*, orcin by *O*, and the specific enzyme which oxidizes orcin by *P*, then an individual containing only *C* and *T* would be colored, *CTO* would be white,

[5] R. A. Gortner: "Studies on Melanin: III. The Inhibitory Action of Certain Phenolic Substances upon Tyrosinase." *Jour. of Biol. Chem.*, Vol. X, No. 2, Sept., 1911.

the cross *CTO* × *CT* would be white (*i. e.*, white would be dominant), the cross *CTO* × *CTP* would be colored (white recessive), while the cross *CTO* × *TP* (both white) would be colored.

It appears, therefore, that Gertner has been able to construct a theory that renders intelligible the behavior of all kinds of white color in inheritance, and to give what seems to be a very plausible chemical explanation of all these cases. We, of course, already understand why the cross of two whites of types *Ct* and *cT* should give color and why both of the latter types of white are recessive to color.

Readers of the NATURALIST are familiar with my contention that in order to show that the chromosomes, as a whole, are not responsible for Mendelian characters, it must be shown that more *independent* dominant characters can be put into a single individual than there are pairs of chromosomes. The fact has been repeatedly cited that more Mendelian characters have been found in *Pisum* than there are pairs of chromosomes, and the claim is made that this disproves the chromosome theory of Mendelism. I have repeatedly shown that this is not the case. There might be a thousand Mendelian characters demonstrated for *Pisum*, but until it is shown that more than six of them are genetically independent, the chromosome theory is not affected thereby. It has heretofore been *assumed* that two factors, each of which when crossed with its absence behaves as a so-called unit character, are genetically independent of each other. This assumption appears to be involved in the "presence-absence" hypothesis. This hypothesis, as usually applied, seems to imply the presence or absence of a particular *body* in the cell, which body, when present *once*, passes into only one gamete, and that there are as many such genetically independent bodies as these are Mendelian factors. When correlation of Mendelian factors occurs, it is assumed to be due to the adhering together of two of these bodies; likewise when the so-called spurious allelomorphism occurs, it is because two of these bodies *repel* each other.

I wish here to repeat what I have often said before, that the fact that two factors each behaves as an allelomorph to its absence *does not prove them to be genetically distinct*. The occasion for this repetition is some pertinent evidence which has just been presented by some exceedingly interesting work of Professor Emerson's.[6] He found that red cob (with white pericarp)

[6] R. A. Emerson: "Genetic Correlation and Spurious Allelomorphism in Maize." 24th An. Rep., Nebr. Agric. Exp. Sta., pp. 59–90.

× absence of red gives the usual Mendelian phenomena of 3 red cob: 1 white in F_2. Likewise, the cross red pericarp (with white cob) × absence of red gives 3 red pericarp: 1 white. Thus each of these types (factors) behaves as an allelomorph to its absence. Hence they should be due to genetically independent genes. But such is not the case; they are allelomorphic to each other.

Some students would say that this is because they *repel* each other. But this explanation does not satisfy in this case, for it can hardly be doubted that red cob in the one case and red pericarp in the other are due to the same cause, acting differently in the two cases. I am of opinion that many similar cases of factors behaving as allelomorphs to their absence will be found to be also allelomorphic to each other. Such cases have usually not been looked for. Quite a number of them have been reported, and I hope some time to be able to bring them all together for reference. Emerson[7] gives a case in beans, in which a variety with green leaves and green pods was crossed with another having green leaves and yellow pods. F_2 consisted of three of the former to one of the latter. Here green was allelomorphic to absence of green. Later he crossed two varieties, one with green leaves and green pods, the other yellow leaves and pods. F_2 consisted of three of the former and one of the latter. Here green was again allelomorphic to its absence. These two crosses apparently show that yellow pods with green leaves and yellow pods with yellow leaves are not genetically distinct. Yet if yellow had been dominant in both these cases, it would have been the usual custom to consider that the two different yellows were independent genetically, because each was allelomorphic to its absence. It would be interesting to know how the two yellows would behave if crossed. It would not be at all surprising to find these two "absences" exhibiting the phenomenon of spurious allelomorphism. The case would be still more interesting if a variety could be found with yellow leaves and green pods.

W. J. SPILLMAN

(To be continued)

[7] *L. c.*